



## DOCTOR OF MEDICINE

### **Development of dual energy X-ray absorptiometry to measure hand bone mineral content for the assessment of rheumatoid arthritis**

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**DEVELOPMENT OF DUAL ENERGY X-RAY ABSORPTIOMETRY TO  
MEASURE HAND BONE MINERAL CONTENT FOR THE  
ASSESSMENT OF RHEUMATOID ARTHRITIS**

**SUBMITTED BY ATULYA A. DEODHAR**

**FOR THE DEGREE OF MD OF THE UNIVERSITY OF BATH**

**1996**

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### **DECLARATION**

**I HEREBY DECLARE THAT THIS THESIS IS ENTIRELY MY OWN WORK. I HAVE PERSONALLY CARRIED OUT MOST OF THE BONE BIOCHEMISTRY MEASUREMENTS AT THE BATH INSTITUTE OF RHEUMATIC DISEASES, BATH AND THE ROYAL CORNWALL HOSPITAL, TRELISKE, TRURO. I HAVE ALSO CARRIED OUT MOST OF THE CLINICAL ASSESSMENTS AS WELL AS THE HAND BONE MINERAL CONTENT MEASUREMENTS ON CONTROLS AND PATIENTS AT THE DUKE OF CORNWALL RHEUMATOLOGY UNIT, TRURO. THE BONE ASHING EXPERIMENTS DESCRIBED IN THIS THESIS WERE CARRIED OUT BY THE SCIENTISTS AT THE CHARLES SALT CENTRE, ROBERT JONES AND AGNES HUNT HOSPITAL, OSWESTRY.**

**ATULYA A. DEODHAR**

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## **DEDICATION**

**I GRATEFULLY ACKNOWLEDGE THE PATIENCE AND  
UNDERSTANDING OF MY FAMILY WHILE I WAS WORKING ON  
THIS PROJECT AND WISH TO DEDICATE THIS THESIS TO HEM  
AND PARIMAL**

## **CONTENTS**

	Page
SUMMARY .....	6
INTRODUCTION .....	8
BONE MASS MEASUREMENT AND BONE METABOLISM IN RHEUMATOID ARTHRITIS: A REVIEW .....	15
DEVELOPMENT AND VALIDATION OF THE METHOD OF MEASURING HAND BONE MINERAL CONTENT BY DUAL ENERGY X-RAY ABSORPTIOMETRY .....	45
MEASUREMENT OF HAND BONE MINERAL CONTENT BY DUAL ENERGY X-RAY ABSORPTIOMETRY IN NORMAL VOLUNTEERS .....	57
MEASUREMENT OF HAND BONE MINERAL CONTENT BY DUAL ENERGY X-RAY ABSORPTIOMETRY IN PATIENTS WITH ESTABLISHED RHEUMATOID ARTHRITIS: A CROSS SECTIONAL AND ONE YEAR LONGITUDINAL STUDY .....	65
TWO YEAR FOLLOW-UP STUDY OF HAND BONE DENSITY AND BONE BIOCHEMISTRY IN EARLY RHEUMATOID ARTHRITIS .....	78
DISCUSSIONS AND CONCLUSIONS .....	97
REFERENCES .....	104



## **SUMMARY**

### **Introduction and Aims:**

It is known that early medical intervention is absolutely vital to improve long term outcome of rheumatoid arthritis (RA). However, further research is required to develop newer outcome measures that can be objectively and reproducibly used early in the course of the disease to predict prognosis. Involvement of bone in RA is common and both generalized and peri-articular osteoporosis occur in RA. Hands are generally the earliest site to be affected in RA and radiological changes in the hands reflect the severity and progression of the disease. The main purpose of this investigation was to develop a method of measurement of hand BMC by dual energy x-ray absorptiometry (DXA) and to evaluate its potential in monitoring disease progression in patients with RA.

### **Materials and Methods:**

Hand BMC was measured by DXA using a Hologic QDR 1000 machine and a modified spine analysis protocol. A customized perspex-aluminium plate was prepared to compensate for the smaller amount of soft tissue and bones in hands and the bone to soft tissue threshold was altered empirically to get a reasonable definition of the bone outline. Nine healthy normal volunteers (3 males, 6 females, age 26-53 years) participated in the initial studies on reproducibility and inter-observer variability. Two pathology human hand specimens were used to investigate the accuracy. To determine the normal ranges of hand BMC, ninety five volunteers (46 males, 49 females, age 20-83 years) underwent hand BMC measurements on both hands. Thirty seven volunteers agreed to be re-scanned to establish the annual rate of change.

We then applied this technique in a cross-sectional study to fifty-six patients (22 males, 34 females age 25-86 years) with RA of varying severity and duration to study the effect of disease on hand BMC, and then in a one year longitudinal study to thirty-nine of the original patients to study the annual rate of change. We then carried out a two year longitudinal study on hand BMC and biochemical bone markers in forty three patient (17 males 26 females age 29-77 years) with early RA (disease duration less than two years).

### **Results:**

The reproducibility (CV) of our technique was 1.3% for hand BMD and 2.3% for hand BMC. In normal volunteers, the median dominant hand BMC was 46.53 grams in males and 30.82 grams in females. It was dependent on the body size and correlated with

hand volume, weight and height. Unlike the BMC in spine and hip, there was no significant correlation between hand BMC and age in volunteers. In the population studied, there was no significant difference between the hand BMC of the dominant and non-dominant hand either in males or in females. After correcting for body size, males had higher hand BMC than females ( $p=0.01$ ). On annual follow-up, male and pre-menopausal female volunteers did not lose any hand BMC, but post-menopausal females did lose significant amount of hand BMC. In the cross-sectional study on patients with established RA (disease duration more than two years), the median dominant hand BMC was 42.7 grams in male patients (7.5% reduced,  $p=0.003$ , when compared to male controls) and 28.4 grams in female patients (7.8% reduced,  $p=0.01$  when compared to female controls). Over a one year period as a group, there was no significant change in parameters of disease activity and in the hand BMC. But the changes in hand BMC in individual patients varied from -33% to +8%.

The hand BMC in male patients with early RA (disease duration less than two years) was 43.5 grams (7% reduction) and 27.7 grams in post-menopausal female patients (8.5% reduction) at baseline. In a two year longitudinal study on this cohort, there was a progressive loss of hand BMC (compared to baseline -4.65% in the first year and -6.4% in the second year) despite significant improvement in other parameters of disease activity. This loss of hand BMC correlated with mean CRP over two years and inversely with serum osteocalcin over the first year. The collagen cross-link excretion remained increased throughout the two year study period.

## **Conclusions:**

We have developed a reproducible method of measurement of hand BMC and have successfully applied it to normal volunteers and to patients with RA. This novel technique is capable of measuring hand BMC changes in patients with early RA even before erosive changes on x-rays are apparent. It therefore has a potential of becoming an investigation of choice in the very early stages of RA to predict future outcome.

## **INTRODUCTION**

## **Rheumatoid Arthritis:**

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease of unknown etiology characterized by symmetric polyarthritis mainly affecting small joints of hands and feet. The first convincing and clear description of the disease was made by Landre-Beauvais in 1800 but the name 'Rheumatoid Arthritis' was coined by Sir Alfred Baring Garrod in 1859. As the evidence for the existence of RA before the year 1500 is sparse and unconvincing, Boyle and Buchanan postulated that RA is a disease of the modern era (Boyle et al 1971). The prevalence and incidence of RA varies between different populations and data from North America and Europe show the overall prevalence rate of seropositive RA to be 1% in Caucasian adults (Lawrence et al 1994). Women are more frequently affected than men at a ratio of 2-3:1 and the prevalence of RA appears to increase with age in both sexes (Lawrence et al 1994).

## **Clinical Manifestations:**

The clinical features of RA can be divided into articular and extra-articular manifestations. Symmetric polyarthritis affecting small joints of hands and feet is the most dominant articular manifestation which usually follows a course of exacerbation or flares and remissions. The natural history of the disease is variable with little joint damage and functional impairment in some patients to progressive irreversible joint damage and significant disability in others.

Extra-articular manifestations include pericarditis, pleuritis, Felty's syndrome, subcutaneous nodules, vasculitis and anaemia. Osteoporosis of the axial and appendicular skeleton is frequently found in patients with RA and has been recognized for a long time (Barwell et al 1865). The generalized bone loss in the axial skeleton has been suggested as one of the extra-articular manifestations of the disease. Localized peri-articular osteoporosis on the other hand, is one of the earliest signs of RA on hand x-rays (Brook et al 1977). The pathogenesis of this juxta-articular loss of bone is not completely understood though locally produced mediators of inflammation are likely to be an important cause (Joffe et al 1991). Anti-rheumatic drugs such as methotrexate and corticosteroids can also contribute to the extra-articular manifestations in patients with RA.

**Treatment:**

The current strategies employed in the treatment of RA are directed towards

1. Alleviation of the signs and symptoms of inflammation
2. Prevention of tissue destruction
3. Preservation of function and
4. Reversal of extraarticular phenomena threatening organ functions such as mononeuritis, pericarditis and lung fibrosis.

These goals are achieved by a multi-disciplinary team approach with special emphasis on patient education, counseling, physiotherapy, occupational therapy and pharmacotherapy.

Pharmacotherapy remains the mainstay of the treatment of RA and is divided in 'first' and 'second line' drugs. The first line drugs include simple analgesics like paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) e.g. indomethacin, naproxen, diclofenac etc. These drugs give immediate relief of pain and inhibit joint inflammation and stiffness but do not influence the natural history of the disease.

The 'second line' drugs are added to the first line agents if the effects of the NSAIDs alone are not sufficient in controlling the disease. These drugs e.g. hydroxychloroquine, d-penicillamine, sulfasalazine, gold salts, azathioprine, methotrexate and cyclosporin are also called 'disease modifying anti-rheumatic drugs' (DMARDs) or 'slow acting anti-rheumatic drugs' (SAARDs). These names suggest that these drugs have the capacity to alter the course of the disease and secondly they have a delayed onset of action, hence their efficacy can only be evaluated after 3 to 6 months. Corticosteroids such as oral prednisolone are also thought to belong to this class of drugs by some authors as they can delay development of erosions (Kirwan et al 1995). More recently, new biologic agents such as anti TNF- $\alpha$  and IL1 receptor antibody have also been successfully used in the treatment of refractory rheumatoid arthritis (Elliot et al 1993).

**Prognosis And Disease Modification:**

Long term outcome of treating RA can be pessimistic with one study of 20 years follow-up showing most patients (54%) either dead (35%) or severely disabled (19%) with only 18% leading a normal life (Scott et al 1987). The apparent failure to influence the prognosis of RA could either be due to use of inappropriate outcome measures in

monitoring therapy or because of the use of ineffective drugs. It is now clear that if we are to avoid such disastrous outcome, early intervention with available treatment modalities is absolutely vital. Fries et al (1996) in a 20 year follow-up study showed that an early and sustained use of DMARDs led to a 30% reduction in long term disabilities as measured by the Health Assessment Questionnaire (HAQ).

However two important questions need to be answered before the early intervention:

1. Which patients should be subjected to these potentially toxic agents, and
2. How early is 'early intervention'.

To answer these two questions we need to understand the prognosis of the patient as the treatment can then be adjusted accordingly. Patients with good overall prognosis could be treated with NSAIDs only or with a less effective but less toxic second line agent. Patients at worse risk with bad prognosis could be treated at the earliest opportunity - even at presentation - with a combination of first and second line agents with careful monitoring of response to therapy.

#### **Prognostic Value of Early Features of Rheumatoid Arthritis:**

To answer which patients would benefit from early intervention, several studies have looked at the features of RA at presentation and its relation to long term prognosis (Flemming et al 1976, Hart et al 1977, Emery et al 1992). Those features indicating a poor prognosis are female sex, older age at onset, involvement of multiple joints, seropositivity, nodule formation, early erosions on x-rays, poor sulfoxidation capacity and those with a conserved sequence of the HLA-DR beta allelic third hypervariable region (the 'shared epitope'). Except for the last two features, the remaining factors can be easily assessed in routine clinical practice. However, as RA is more common in older population and in females, some of these factors lack specificity (e.g. female sex and older age at onset) and other important factors lack sensitivity and may not be existent at the time of presentation (e.g. presence of nodules and erosions on x-rays). Further research into this area is therefore urgently required to develop newer markers that can be objectively and reproducibly used early in the course of the disease to predict prognosis.

### **Process Versus Outcome Variables:**

A distinction needs to be made between different types of variables measured in the study of RA. Process variables measure the actual disease activity and may vary widely during the course of the disease. The example of process variables are early morning stiffness, number of swollen and painful joints and laboratory measures of acute phase reactants. Outcome variables on the other hand, measure the effect of the disease over a defined period of time. Some examples of outcome variables include functional outcome as measured by Health Assessment Questionnaire (HAQ) (Fries et al 1980) and radiological outcome measured by various methods of scoring x-rays (Larsen et al 1977, Sharp et al 1985). Ideally the variables measuring process (disease activity) should predict the outcome, though multiple measurements of process variables over a period of time are needed to achieve this. Outcome variables have their own limitations. Their use over a short term is not ideal as they are insensitive to acute change.

### **Radiological Methods of Measuring Outcome in RA:**

Simple joint radiographs in patients with RA measure three aspects: peri-articular osteoporosis, loss of joint space and the appearance and progression of erosions. Various methods of scoring radiographs (Larsen et al 1977, Sharp et al 1985) are however time consuming, need interested and trained observers and are open to subjective interpretation. The other major problem afflicting these methods is that they are insensitive to the early changes of peri-articular osteoporosis. Furthermore, identification of erosions depends on the quality of the radiograph and hand position and there is no consensus whether appearance of new erosions is more important than the changes in the size of the existing ones.

These so-called 'gold standard' radiographic outcome measures can therefore become 'fools gold' in inexperienced hands (Brower 1990).

### **Measurement Of Bone Mass In RA:**

The advent of measurement of bone mineral density (BMD) by various methods (for review see Wahner et al 1994) has opened exciting new possibilities of finding new candidates for measuring outcome in RA (Sambrook et al 1986, 1987, Laan et al 1992). Dual energy x-ray absorptiometry (DXA) has superseded other methods because of its superior precision, accuracy and safety of minimum radiation involved. Unlike the x-ray

scoring methods, this technique is objective and the results depend minimally on the operator.

The rationale for using bone mass measurements as outcome measure in RA are many. Apart from the early signs of peri-articular osteoporosis, generalized skeletal effects of the disease have been recognized more recently (Verstraeten et al 1986, Sambrook et al 1986, 1987, Compston et al 1988). Major etiological factors leading to this bone loss include the direct effect of inflammatory mediators, decrease in physical activity and corticosteroid treatment (Als et al 1985, Bhalla et al 1991, Laan et al 1992). Recent longitudinal studies have shown significant generalized bone loss even within the first few months of disease in patients with RA that correlated with disease activity (Gough et al 1994, Shenstone et al 1994). Bone mass therefore appears to be a variable that sensitively reflects the disease activity and severity. With the growing interest in the field of osteoporosis, the DXA machines are becoming more easily available in district general hospitals, making bone mass measurement in patients with RA a realistic possibility.

#### **Hand Bone Densitometry In RA:**

Most studies on skeletal changes in RA have been limited by the available techniques and investigate the total body calcium or the bone mass at the lumbar spine, femoral neck or the distal radius (Reid et al 1982, Sambrook et al 1986, 1987, Verstraeten et al 1986, Compston et al 1988, Gough et al 1994, Shenstone et al 1994). However these may not be the ideal sites to monitor rheumatoid arthritis. The hand with its multiple joints and periarticular bone involvement in RA, combined with the functional effects of disease on its usage may act as a site for composite assessment of overall disease progression. Longitudinal studies of hand bone mass measurements may therefore be a better way to assess the severity and be more sensitive in measuring the progression of RA and predicting response to treatment.

#### **Aim And Contents Of This Thesis:**

The main purpose of this thesis is to develop a method of measurement of hand bone mineral content (BMC) by DXA and to evaluate its potential in monitoring disease progression in patients with RA.

The literature on bone metabolism and bone mass measurement in RA is reviewed in the next chapter. The development of the method to scan the hand by DXA is then



described in chapter 3, including the modifications in the Hologic spine protocol needed to analyze hand BMC. The accuracy and reproducibility of the new method of hand bone densitometry is also described. After the development of the method, we investigated the normal ranges of hand BMC as well as the causes of variability in hand BMC in healthy male and female volunteers (chapter 4).

We then applied this new method to patients with rheumatoid arthritis of varying duration and severity to assess the effects of the disease on hand BMC as well as to investigate the changes in hand BMC over a one year period (chapter 5). In a chronic disease like rheumatoid arthritis, from the clinician's and the patient's point of view the most important time to predict prognosis is early in the course of the disease before erosions develop. Furthermore, previous radiological studies on the axial skeleton in RA suggest that more pronounced changes in bone mass occur early in the course of the disease. We have therefore looked in greater detail at the changes in hand bone mass in a cohort of patients with 'early RA' (disease duration less than 2 years) over a period of two years and have compared them with biochemical indices of bone metabolism (chapter 6). Finally, the discussion and conclusions are offered in chapter 7.

**BONE MASS MEASUREMENT AND BONE METABOLISM**  
**IN RHEUMATOID ARTHRITIS: A REVIEW**

## **INTRODUCTION:**

Involvement of bone in rheumatoid arthritis (RA) was first described by Barwell in 1865 and since then it is known that both generalized and peri-articular osteoporosis occur in RA (Kennedy et al 1977). Juxta-articular osteoporosis is one of the earliest radiological abnormalities in RA (Brook et al 1977). With the increasing availability of non-invasive radiological techniques of measuring bone mass (e.g. single and dual photon absorptiometry and dual energy x-ray absorptiometry) several studies have demonstrated generalized bone loss in patients with RA (Als et al 1985a, Sambrook et al 86, 87, 89, Reid et al 1986b, Verstraeten et al 1986, Cooper et al 1988, O'Malley et al 1989, Butler et al 1991 Laan et al 1992a, 1993a).

The pathogenesis of this localized and generalized bone loss is not clear. Juxta-articular bone loss is thought to be due to a local increase in vascularity, direct invasion by pannus as well as related to the mediators of inflammatory process in the joint (Joffe et al 1991). In the pathogenesis of the generalized bone loss, several factors including circulating cytokines produced by the inflammatory process (Kaplan et al 1987, Alwan et al 1988, Eastgate et al 1988) and reduced mobility due to functional impairment (Fogelman et al 1986, Woolf et al 1991) are proposed as the important etiological factors. Various drugs like glucocorticoids, and more recently methotrexate, used in the therapy of RA are implicated in the localized and generalized bone loss (Laan et al 1992b, Preston et al 1993). To try and resolve these issues, bone turnover in RA has been studied by new biochemical assays of bone metabolism (Sambrook et al 1985, Gevers et al 1986, Weisman et al 1986, Seibel et al 1989, Pietschmann et al 1989) and also by bone histomorphometry (Shimizu et al 1985, Van Soesbergen et al 1986, Mellish et al 1987, Compston et al 1994). The ends of the spectrum are clear. Patients with longstanding destructive and disabling rheumatoid disease have generalized osteoporosis (Als et al 1985a) with increased risk of fractures (Hooyman et al 1984) and early rheumatoid disease without functional impairment is associated with peri-articular osteoporosis (Kennedy et al 1977). There have been controversies on whether all or only some patients with RA are affected by generalized osteoporosis, the comparative effects of various etiologically agents, the dose of corticosteroids that causes or does not cause bone loss and the clinical significance of this (Sambrook et al 1986, Woolf 1991, Laan et al 1992a, Bhalla et al 1992). Some in fact have suggested that steroids, by reducing the disease activity and improving mobility, stop bone loss (Sambrook et al 1989).

This chapter reviews the measurement of appendicular and axial bone mass by densitometry, and the assessment of bone metabolism using biochemical markers as well as histomorphometry in patients with RA. The important factors in the pathogenesis of bone loss in RA are also discussed.

### **Potential Problems In Interpretation Of Results:**

Before comparing different studies, it is important to recognize the potential problems in data interpretation. These problems can be related to:

1. Nature of variables used (e.g. Process V Outcome variables)
2. Study design (e.g. Cross-sectional V Longitudinal)
3. Methods and their limitations.

Bone loss in rheumatoid arthritis can be monitored by indicators of disease 'process' as well as indicators of 'outcome'. Certain biochemical markers of bone turnover (e.g. osteocalcin and urinary cross-links) reflect acute changes in bone metabolism and hence measure the 'process' of bone loss. Bone mass in an individual is a product of the genetic background and the effect of internal (e.g. hormonal status, presence of RA) and external (e.g. diet, exercise) environment. Measurement of bone mass by DXA thus reflects these multiple factors as well as many years of disease activity, functional capacity and cumulative effect of medication like corticosteroids. Bone mass measurement is therefore likely to be an 'outcome' rather than a 'process' variable.

Many studies are cross-sectional but this design does not allow for risk factors that may have changed over time. Inactive disease at assessment does not exclude the effect of previous disease activity on bone mass. One therefore needs to be cautious with claims of correlation between measurements of disease activity or biochemical markers of bone metabolism and the bone mass.

The third important problem in comparing results of various studies lies with the diverse methods and sites of bone density measurement (Table 1) as well as use of various assays in the measurement of bone metabolism. The results obtained by different methods are not always comparable, for example, the correlation between bone mass at an appendicular site such as the distal radius with the bone mass at an axial site such as femoral neck or lumbar spine is poor, both in normal and in osteoporotic persons

(Wahner et al 1994). Also, diseases (e.g. rheumatoid arthritis and osteoarthritis) and drugs (e.g. corticosteroids) affect different parts of the skeleton (e.g. axial versus appendicular or trabecular versus cortical bone) with varying severity. It may therefore be erroneous to claim 'generalized osteoporosis' looking at appendicular bone mass. Careful attention to the methodology is vitally important in interpretation and comparison of results.

Biochemical markers of bone turnover reflect changes in the entire skeleton, which may not give information about localized osteoporosis in diseases like RA. There is as yet no 'gold standard' method for most of the new biochemical assays and different assays may give conflicting results in the same individual (Eastell et al 1994). Diurnal variation in levels of some of the markers (e. g. urinary collagen cross-links) and effects of various storage methods (e.g. serum osteocalcin can partially breakdown in peptides at -30°C.) also need to be considered when comparing different studies. It is also difficult to compare bone formation (serum markers) with bone resorption (usually urine markers). Histomorphometry involves bone biopsy, which limits its use in longitudinal studies. Biopsies from different parts of the skeleton (peri-articular versus iliac crest) give limited site specific information which has a poor precision.

### **RADIOGRAPHIC MEASUREMENTS OF BONE MASS IN RA:**

#### **Plain Radiographs:**

Plain radiographs of the peripheral joints (mainly hands and feet) are frequently used for diagnosis and for assessment of progression in RA (Scott et al 1985). Out of the three diagnostic features of rheumatoid joint damage, namely peri-articular osteoporosis, joint space narrowing and erosions, two are directly related to loss of bone. To monitor these changes, many radiographic scoring systems have been developed which need single PA film of the hands and wrists (Sharp et al 1971 and 1985, Larson et al 1977, Bluhm et al 1983). All scoring systems take into account joint space narrowing and osseous defects either together (Larsen's method) or separately for individual joints (Sharp's and Bluhm's method).

Over the years, many outcome studies have used these scoring methods to monitor disease progression. However many problems still exist with most of these scoring systems (for review see Brower 1990) because of the technical and interpretational

variables involved. The global scoring method of Larsen et al (1977), where a single score is given for all the radiological abnormalities in the joint under consideration, and the detailed scoring method of Sharp et al (1985), where a separate score is given to each radiological abnormality in the joint concerned, have the problem of poor sensitivity to monitor change. These methods can be either imprecise and coarse or too cumbersome and time consuming. They require interested and trained observers and are not good at quantifying early changes before erosions.

### **Radiogrammetry:**

Radiogrammetry (Burnett et al 1960) is a technique of measuring metacarpal bone mass using hand x-rays and Vernier calipers. Measurements of inner and outer diameters at the midshaft of the right or left second metacarpal is used to calculate the metacarpal area and the bone mass. The manual method (Dequeker et al 1976) has a large coefficient of variation (Bloom et al 1980) and the automated method (Kalla et al 1989) requires a digitizer interfaced with a PC. Using this automated method of radiogrammetry, in a study of 12 male and 69 female patients with RA, Kalla et al(1991) showed improvement in the metacarpal bone mass in a subgroup of patients (n=32) below age 50, with slow acting anti-rheumatic drugs.

The advantages of radiogrammetry include use of comparatively simple equipment with ordinary hand x-rays. However it can only be used on metacarpals and needs interested observers. DXA has surpassed radiogrammetry as a standard investigation of choice for monitoring osteoporosis.

### **Microfocal Radiography And Magnetic Resonance Imaging (MRI):**

Radiographic magnification using a special machine with a microfocal x-ray tube is said to be more sensitive in detecting erosive disease than is conventional radiography (Resnick et al 1989). However positioning is difficult and only a small area can be imaged on one film. The use of MRI in evaluating RA is currently in a period of development. At present the use of MRI in imaging bone is very limited and its use is therefore inappropriate is demonstration and documentation of bony changes (Brower 1994).

### **Bone Densitometry In RA:**

Studies on bone mass measurement in RA focus either on the generalized loss of bone measured in the lumbar spine or femoral neck or study the local osteopenia in the appendicular skeleton like the distal radius or hand. Table 2 summarizes these studies and their conclusions. For this review we have separated the studies on axial (and total body) bone mass from those on appendicular skeleton in patients with RA.

### **Bone Densitometry Of The Appendicular Skeleton:**

Single photon absorptiometry (SPA) was one of the earliest techniques to become commercially available for bone densitometry. Many of the earlier studies used this technique to assess the effect of RA on bone mass by measuring distal radius BMD. However, there are two main problems in speculating about generalized skeletal changes based on bone densitometry results of the distal radius. As explained earlier, subsequent studies have shown that in normal volunteers and in patients with osteoporosis, the correlation between bone mass at an appendicular site such as the distal radius with the bone mass at an axial site such as femoral neck or lumbar spine is poor (Wahner et al 1994). In patients with RA, the 'peri-articular' nature of the distal radius may also affect the results. Depending on the wrist's direct involvement in the rheumatoid process, the changes in the distal radius may be more or less pronounced, leading to erroneous conclusions about general skeleton. A recent study (Peel et al 1994) on total body BMD measurement in patients with RA highlights the differential effect of RA on different parts of the skeleton. When compared to age matched normals, the patients with RA had 11% lower bone mass in the lumbar spine, though 23% lower bone mass in the hand. In a cross-sectional study, Als et al (1984) measured the distal radius BMD by SPA in 129 patients with RA. They found that all patients had lower BMD as compared to normals (84% of normal) and those on oral corticosteroids had even further reductions (73% of normal). Verstraeten et al (1986) found reduced bone mass at the distal radius in 64 patients with RA than controls though they did not find any effect of corticosteroids. In a longitudinal study over two years in 14 patients with RA, Sambrook et al (1990) reported trabecular bone loss (6.1% per annum) in distal radius when measured by computed tomography densitometer using <sup>125</sup>I isotope.

### **Hand Bone Densitometry:**

Hands and feet are generally the earliest sites to be affected in RA (Flemming et al 1976) and radiological changes in the hands reflect the severity and progression of the

disease (Fries et al 1986). It is therefore logical to measure the bone densitometry in the hands of patients with RA.

Pye et al (1990) devised a method of hand bone densitometry using DXA. They modified the lumbar spine software of the Hologic DXA machine and used a perspex aluminium plate to compensate for the small soft tissue and bone thickness in hands. In 70 postmenopausal women with corticosteroid treated RA, Peel et al (1994) showed that hand BMD correlated with BMD at other sites. They however could not find any effect of disease duration, disability or steroid therapy on hand BMD. In this thesis, I describe our experience with this technique in greater details in further chapters.

### **Bone Densitometry In Axial Skeleton:**

Before the advent of dual photon or dual energy x-ray absorptiometry, measurement of total body calcium (TBC) by neutron activation analysis gave some idea about the total bone mass. Reid et al (1982) measured the TBC by neutron activation analysis in 63 patients with RA and showed that they had lower TBC than normals. 31 patients who had used oral corticosteroids in the dose of 6 mg per day for up to 5 years had a further reduction in TBC as compared to those who did not use corticosteroids. In a longitudinal study (Reid et al 1986a) they showed that patients lost 3.7% calcium per annum. When these patients were divided according to their steroid dose, it was noted that oral corticosteroids in a dose of less than or equal to 5 mg per day was protective. Patients on this low dose did not lose any TBC whereas patients not taking corticosteroids or those taking higher than 5 mg per day, lost significant amount of TBC.

Further studies on axial skeleton in patients taking oral corticosteroids face several problems because of the number of factors involved that can potentially influence the bone mass. Apart from the possibility that patients receiving oral corticosteroids are likely to have more severe disease and they are likely to be more disabled, these studies had to account for the steroid dose, daily and cumulative, as well as duration of steroid treatment. In a cross-sectional study, Sambrook et al (1986) has studied the effect of corticosteroids on bone mass in 84 female patients with RA. 44 patients had taken oral corticosteroids in the mean dose of 8 (range 2.5-16.8) mg per day for an average of 7.5 years. They found that as compared to a control group, there were significant reductions in the spinal (9.6%) and femoral neck (12.2%) BMD in patients treated with corticosteroids as well as in those who had not received corticosteroids (reductions of 6.9% in lumbar spine and 8.9% in the femoral neck). The differences between these two



groups however did not reach statistical significance. They therefore concluded that low dose prednisolone was not associated with increased risk of osteoporosis. Further cross-sectional and longitudinal studies by the same group (see Table 2) seem to confirm this finding. In a cross-sectional study (Sambrook et al 1987) on 111 patients, they reported that there was no difference in either the spinal or femoral neck BMD between 44 female patients taking a mean daily dose of 8.5 mg prednisolone and 40 female patients not taking corticosteroids. 12 male patients on a mean daily dose of 10.3 mg however had significantly lower spinal BMD (exact values not given) when compared with 15 others who did not receive oral steroids. On simple or multiple regression analysis, the mean daily dose, the total cumulative dose and the duration of therapy with prednisolone did not correlate with the lumbar spine or femoral neck BMD. Laan et al (1992b) on the other hand, have reported completely opposite results using QCT to measure spinal BMD. In a cross-sectional study of 74 patients with RA, they found that 30 patients using oral corticosteroids (mean 7 mg per day for 6 years) had 31% reduction in the trabecular BMD and 37% reduction in the cortical BMD in the spine compared to those who did not use them. In a further double-blind placebo controlled prospective study they found that prednisolone 7.5 mg per day for 20 weeks led to a partially reversible mean bone loss of 8.2% in the trabecular bone of the lumbar spine (Laan et al 1993b). After discontinuation of the therapy and within 24 weeks, these patients regained 5.3%. Bone mass was not lost in 20 patients who did not use oral prednisolone over this period.

The apparent failure to demonstrate the detrimental effect of corticosteroids on the bone mass in patients with RA in various studies by Sambrook et al is difficult to explain. Close scrutiny of the results show that patients taking oral CS in most of these studies did have lower bone mass as compared to their non-user counterparts, though this difference failed to reach statistical significance. One possible explanation could be that DPA, the technique used by Sambrook et al, is not as sensitive as DXA or QCT - techniques used in later studies by Laan et al. QCT can further separate trabecular and cortical bone in the spine and gives a true volumetric bone density as opposed to Areal density in DPA. QCT is therefore more accurate than DPA.

Disease duration appears to be an important determinant of bone loss in RA. It is known that the natural history of radiological progression in RA is one of an initial rapid rate of damage followed by a gradual reduction in the speed of progression (Sharp et al 1985). Two recent longitudinal studies (Gough et al 1994b, Shenstone et al 1994), both using DXA as the measurement technique, showed that patients with RA lose lumbar spine

BMD mainly in the early part of their disease. Gough et al studied 148 patients over 3 years period and found that the annual rate of loss of BMD in the lumbar spine was 0.2% for males and 1.3% for females. When the patient population was divided according to their disease duration, female patients with early RA (disease duration < 1 year) were found to have lost 2.5% (no results given for early RA males). Shenstone et al (1994) found that 16 patients with disease duration of < 6 months lost 3.9% femoral neck BMD compared to 0.2% loss in 51 patients with longer disease duration. The low sensitivity of the DPA technique to measure change in BMD may also explain findings of another longitudinal study (Sambrook et al 1990) which found no significant reduction in the lumbar spine bone mass over two years in 17 female patients with early RA (mean disease duration 1.2 years) as compared to 18 age matched controls. These female patients however lost significant amount of distal radial trabecular bone (11.1% in the first year and 1.4% in the second year), although both wrist joints were affected by rheumatoid process in all patients.

#### **CONCLUSIONS ON BONE DENSITOMETRY STUDIES IN PATIENTS WITH RA:**

1. Patients with RA have lower bone mass as compared to normals in appendicular and axial skeleton.
2. Longitudinal studies show that the predominant loss of bone mass occurs early (within the first year) after the disease onset.
3. Oral corticosteroids more than 5 mg per day are associated with significant loss of bone mass. The effect of corticosteroid doses less than or equal to 5 mg per day is unclear.

#### **BIOCHEMICAL MARKERS OF BONE TURNOVER IN RA:**

Bone is a metabolically active living tissue undergoing constant remodeling to maintain its structural integrity. Bone remodeling occurs in discrete remodeling units, where a cavity on the bone surface is eroded by osteoclasts. After a quiescent phase, osteoblasts replace an equivalent amount of bone. During bone resorption, constituents of bone matrix, mainly collagen, find their way into the circulation and then are excreted in the urine through the kidneys. Markers of bone resorption include plasma tartrate resistant acid phosphatase, urinary hydroxyproline, pyridinoline and deoxy-pyridinoline which are the breakdown products of collagen cross-links (Adachi 1991). Osteoblasts

produce alkaline phosphatase and osteocalcin, the best known markers for bone formation. However, as explained above, there are no gold standard assays available as yet and the accuracy and precision between different assays within and between laboratories can be considerable (Eastell 1994).

In the last ten years many studies have investigated the usefulness of these markers in monitoring the bone changes in RA (Sambrook et al 1985, Weisman et al 1986, Seibel et al 1989, Pietschmann et al 1989, Marhoffer et al 1991, Kollerup et al 1994). Table 3 summarizes the studies discussed in this review.

### **Biochemical Markers Of Bone Formation:**

Serum total alkaline phosphatase has been a readily available biochemical test for many years. Apart from bone; liver, placenta and intestine also produce alkaline phosphatase isoenzymes (Peel et al 1992). The bone isoenzyme is secreted by osteoblast and its measurement is therefore a marker of bone formation. Unfortunately, levels are only elevated when bone turnover is very high as in Paget's disease and correlation with high, normal or low bone turnover in osteoporosis is poor (Adachi et al 1991). Total alkaline phosphatase is found to be raised in patients with RA (Weisman et al 1986, Verstraeten et al 1986), though studies on bone specific alkaline phosphatase in RA are not available.

Osteocalcin or bone GLA protein (BGP) is a vitamin K dependent non-collagenous protein in adult bone (Eastell 1994) produced by osteoblasts. Studies in patients with RA have demonstrated variable osteocalcin (OC) levels. Sambrook et al (1985) studied 17 female patients with early RA (Disease duration mean 1 year). They found no difference in the serum OC levels in early RA patients and controls. They however did not give any information about use of corticosteroids by patients in this study, though this factor could influence serum OC levels (Weisman et al 1986). Weisman et al (1986) showed that in 81 patients with RA, only 38 patients on oral corticosteroids had significantly reduced serum OC levels, whereas the steroid non-users had normal OC levels. This finding was later confirmed by Ekenstam et al (1986) who showed that 20 mg of oral prednisolone for one week is sufficient to cause a reversible reduction in osteocalcin levels. In their study, 36 patients with RA and 23 patients with sero-negative spondyloarthropathy had significantly low levels of OC even before taking oral corticosteroids. They therefore concluded that inflammatory arthritis leads to reduced bone turnover and bone formation is further reduced by oral corticosteroids.

Two further studies however showed raised OC levels in patients with RA (Magaro et al 1989, Marhoffer et al 1991) which were found to correlate with the disease activity. Magaro et al found that in 42 patients, those with active RA had 82% higher OC levels than patients with inactive RA and Marhoffer et al demonstrated increased levels of OC in older patients (age > 65 years) with new onset RA. These patients however had more active disease (as defined by raised ESR) and over one year, the disease activity and serum OC both dropped.

### **Biochemical Markers Of Bone Resorption:**

Studies on markers of bone resorption such as pyridinoline and deoxy-pyridinoline show more uniform results (see table 3). Pyridinoline (PYD) is the main cross-link between the collagen fibrils in cartilage and the most abundant cross-link in bone. Deoxy-pyridinoline (DPD) however is bone specific, being derived from type 1 collagen in bone and is not found in cartilage. There is considerable diurnal variation in the rate of cross-link excretion (Peel et al 1992) and it is necessary to collect the urine samples in a uniform manner, at the same time of the day. A study by Robins et al (1986) showed significantly high levels of PYD excretion in 11 patients with RA compared with 20 patients with OA. No information is however given about use of corticosteroids, menopausal status of the patients as well as the timing of urine collection. Seibel et al (1989) et al showed that both PYD and DPD excretion in urine was significantly raised in RA, but urinary PYD levels, which is a marker of cartilage and bone degradation, correlated with clinical measures of joint involvement (Lansbury joint index, pain score and number of joints involved) as well as biochemical variables of inflammatory activity (CRP and ESR). Black et al (1989) and Gough et al (1994a) have also shown that patients with active disease have higher urinary PYD excretion.

DPD which is a bone specific marker, is also found to be raised in patients with RA though it does not correlate with disease activity (Seibel et al 1989, Black et al 1989, Gough et al 1994a).

Hydroxyproline, another marker of collagen breakdown is found in many types of collagen apart from bone. Its excretion is affected by collagen breakdown in the skin, tendons, blood vessels etc. and it is also released during activation of the complement cascade as a breakdown product of C1q. This is an important confounding factor in the inflammatory arthropathies. Hydroxyproline excretion is also affected by dietary intake of gelatin, necessitating collection of fasting urine (Uebelhart et al 1990). Tartrate resistant

acid phosphatase (TRAP) is another marker of bone resorption (Eastell 1994). However at present the assay system of TRAP is either indirect or non-specific and the lability of the enzyme does not allow reliable measurements on frozen samples (Adachi 1991).

## **CONCLUSIONS ON BIOCHEMICAL MARKERS OF BONE TURNOVER IN RA:**

1. Markers of bone formation give conflicting results in patients with RA. It is not clear whether the osteoporosis in RA is secondary to reduced rate of bone formation or whether the rate of bone formation is raised in RA, though not sufficient enough to keep pace with the rate of resorption.
2. Markers of bone resorption show more uniform results with significant increase in patients with RA as compared to controls. Patients with active disease have higher levels than patients with non-active disease.

## **BONE HISTOLOGY AND HISTOMORPHOMETRY IN RA:**

Bone histology has been studied in RA by either iliac crest biopsies (representing generalized changes in the skeleton) or by a sample of bone taken from an operation site (usually representing peri-articular bone changes). The invasive nature limits its use in longitudinal studies and despite increasing sophistication, the methods have poor precision (Eastell 1994).

Wordsworth et al (1984) found evidence of osteoporosis in 10 out of 25 consecutive in-patients with RA on histological grounds, judged by a reduction in number and thinning of trabeculae. The bone biopsies were either taken from iliac crest or from the operation site. 14 patients had taken corticosteroids in a dose less than or equal to 10 mg per day for up to 24 years. The dietary intake of vitamin D was found to be well below the recommended dose in all 25 patients, but there were no histological changes of osteomalacia. Mellish et al (1987) studied 48 patients of rheumatoid arthritis none of whom had received corticosteroids. They compared the iliac crest trabecular bone histology with healthy controls matched for age and sex. The evidence of significant trabecular thinning was noted in female patients only. 2 studies by Compston et al (1989, 1994) show significant reduction in the eroded perimeter, mean and maximum eroded depth and cavity area in patients with non-steroid treated RA. They therefore concluded that there is reduced bone formation and low bone turnover in RA as compared to healthy controls.

Shimuzu et al (1985) on the other hand found exactly opposite results on biopsies from juxta-articular bone removed during joint surgery. 20 areas from 12 patients with RA were studied and compared with 14 areas from 6 OA patients. 25% of the RA patients had taken low dose oral corticosteroids (< 4 mg per day). They found increased bone formation as suggested by the increase in the percent of active osteoid surface as well as increase in bone resorption as evidenced by increase in percent resorptive surface and number of osteoclasts. They therefore concluded that there is increased turnover of juxta-articular bone in RA. This apparent discrepancy in the findings of these two investigators could be partly explained by the different sites used to obtain bone biopsies in these studies. Shimuzu et al studied the peri-articular bone changes as opposed to Compston et al, who studied generalized bone changes by iliac crest biopsies.

### **CONCLUSION ON BONE HISTOMORPHOMETRY IN RA:**

It is possible that the peri-articular osteopenia in RA is related to increased bone turnover locally, whereas the generalized osteoporosis could be due to a global negative remodeling balance.

### **DETERMINANTS OF BONE MASS IN PATIENTS WITH RA:**

Determinants of bone mass have been sought by either calculating correlation coefficients between bone mass and a single suspected determinant or by multivariate techniques (Sambrook et al 1987, Laan et al 1993a). The former method is of limited value since bone mass in patients with RA is under multifactorial influence.

#### **Sex And Menopausal Status:**

There has been no uniform effect of sex on differences in bone density in RA and controls. In a cross-sectional study, O'Malley et al (1989) et al found a reduction in spinal BMD in male patients with RA compared to normals whereas the female patients showed more pronounced reductions in femoral neck compared to normals. Laan et al (1993a) found both male and female patients with RA had reduced bone mass as compared to sex-matched normals to more or less same extent. The study of Als et al (1985b) looked at the effect of menopausal state and oral corticosteroids (mean dose 8 mg) in 97 patients with RA. They found that only in pre-menopausal patients was the bone mass significantly affected by corticosteroid treatment. In post-menopausal RA

patients, the bone loss resulting from menopause and the disease itself, was not accelerated by oral corticosteroids. Butler et al (1991) on the other hand found that the adverse effects of RA on bone mass are accentuated in the post-menopausal state.

#### **Disease Duration:**

Disease duration seems to be an important factor to be considered when studying the effect of RA on bone mass. Two recent studies by Gough et al (1994b) and Shenstone et al (1994) have confirmed that the deleterious effect of RA occurs mainly in the first year of the disease. There were significant differences in the rate of loss of bone between patients with disease duration of less than 1 year and those with disease duration of more than 1 year. In the study by Shenstone et al, patients with disease duration of less than 6 months lost 3.9% femoral neck bone density compared to only 0.2% loss in the remainder of cohort in 67 patients with RA.

#### **Disease Activity:**

Cross-sectional study design is not suitable to assess the effect of disease activity on bone mass. Several longitudinal studies have shown that patients with high disease activity lose more bone density than those with inactive disease. The disease activity is however measured differently in various studies. Shenstone et al (1994) used the 'Stoke Index' (Davis et al 1990) which is a composite algorithm incorporating joint count, duration of early morning stiffness, ESR, Ritchie index and CRP, and found that the lumbar spine BMD changes correlated with the initial Stoke Index but not the mean Stoke Indices. Gough et al (1994b) stratified their patients into those with CRP of < 20 and those with CRP of > 20 mg/dl. Patients with active disease showed significantly greater BMD losses at the femoral neck, Ward's triangle and lumbar spine compared to patients with inactive disease. In a longitudinal study Laan et al (1993a) used several measures of disease activity and found that the mean ESR in 6 months before BMD measurement was negatively associated with the BMD in the hip and in Wards triangle. Other parameters of disease activity did not show any correlation with BMD.

Biochemical indices of bone metabolism have also been shown to be raised in active disease. Raised serum osteocalcin levels (cross sectional study by Magaro et al 1989, longitudinal study by Marhoffer et al 1991) and increased urinary excretion of PYD in patients with active disease (Gough et al 1994a) have been described. In the study by Gough et al, urinary PYD levels (measured only once within the first three months of the study) correlated with disease activity (mean of four CRP values measured at entry, 3, 6



and 12 months) and also with loss of BMD in the femoral neck (measured twice, one year apart). This study design is obviously not ideal and it therefore reduces the impact of the conclusion that disease activity leads to high bone turnover and consequent bone loss. As the biochemical markers reflect changes in entire skeleton, it is not possible to know whether this accelerated bone turnover is limited to juxta-articular sites as suggested by the histomorphometric studies by Shimizu et al (1985).

### **Reduced Mobility:**

It is known that the level of weight bearing activity is an important determinant of bone mass and patients of RA may have reduced general mobility secondary to their disease. Sambrook et al (1987) found that reduced physical activity is an important factor in axial bone loss in RA. They use the Framingham activity index (Kannel et al 1979) and found that in a multiple linear regression analysis, the association of physical activity persisted with the femoral BMD but not with the lumbar spine BMD. Parameters of disease activity were however not included amongst the variables considered in this analysis. Functional capacity evaluated either by Steinbrocker classification or health assessment questionnaire is a better and more objective way of assessing physical activity. Gough et al (1994b) stratified their patients in to those with HAQ scores of  $> 8$  (HAQ index of  $> 1$ ) and those with HAQ score of  $< 8$  (HAQ index of  $< 1$ ). The more disabled patients (HAQ  $> 8$ ) had significantly greater loss of bone at the lumbar spine, femoral neck and Wards triangle. They however do not make it clear whether this effect of functional incapacity is independent of disease activity or not. In a longitudinal study, Laan et al (1993a) found that the mean HAQ score in the 18 months before BMD measurement was negatively associated with BMD in hip only. This was independent of the disease duration and the mean disease activity measured by ESR. In a cross-sectional study by Black et al (1989), urinary PYD levels correlated inversely with grip strength in 19 patients with RA, indicating that patients with reduced upper limb function (and perhaps mobility) have increased bone resorption.

### **CYTOKINE PRODUCTION IN RA AND THEIR EFFECT ON BONE:**

The adult human bone maintains its strength as a result of continuous cellular activity of bone resorption and bone formation. The activities of these cells are closely regulated and follow a well defined complex sequence termed the bone remodeling cycle (for review see MacDonald et al 1993). This requires both site and cell specific signals capable of initiating and promoting the recruitment and proliferation of appropriate cells

at the appropriate time. This is mediated through cytokines, which are biologically active glycoproteins produced by monocytes, macrophages and T lymphocytes. Over the last decade, a large evidence is accumulated defining roles of different cytokines in the control of bone cell activity (MacDonald et al 1992). We will first describe the role of cytokines in the control of bone remodeling cycle and then describe the evidence suggesting that abnormal cytokine production may be relevant in the pathogenesis of rheumatoid bone disease.

### **Cytokines And Bone Resorption:**

Cytokines acting on bone resorption can be divided into two main groups:

1. Cytokines that stimulate bone resorption: Interleukin 1 (IL-1) and Tumour necrosis factor alfa (TNF $\alpha$ ).
2. Cytokines that inhibit bone resorption: Interferon gamma (IFN $\gamma$ ) & Interleukin 4 (IL-4).

IL-1 remains the most potent cytokine that leads to proliferation and differentiation of osteoclast precursors to osteoclasts. It has a stimulatory action on mature osteoclast activity. Both these effects of IL-1 lead to significant bone resorption at a very small concentration. TNF $\alpha$  has an action very similar to that of IL-1. It acts as a growth factor for osteoclast precursors which are from the monocyte - macrophage lineage (MacDonald et al 1992). The action of IL-1 and TNF $\alpha$  is thought to be synergistic on osteoclasts. Three other cytokines namely, Granulocyte macrophage colony stimulating factor (GM-CSF), Macrophage colony stimulating factor (M-CSF) and Interleukin 6 (IL-6) are thought to be "permissive" cytokines. They help in bone resorption by maintaining adequate numbers of early osteoclasts precursors on which TNF $\alpha$  and IL-1 act (McDonald et al 1992).

Termination of osteoclast activity is also thought to be regulated by two cytokines. IFN $\gamma$  decreases the number of osteoclasts by reducing the recruitment of osteoclasts precursors rather than by having a direct inhibitory effect on mature osteoclasts (MacDonald et al 1992). IL-4 also inhibits bone resorption though the mechanism of its action remains unknown.

### **Cytokines And Bone Formation:**

In the bone remodeling sequence, bone formation is divided into two stages:

1. Recruitment and differentiation of osteoblasts
2. Bone matrix production and mineralization

Cytokines are known to act on both these phases.

IL1 and TNF $\alpha$ , which stimulate osteoclast formation and activity as explained above, also have a stimulatory effect on osteoblast proliferation. Once the osteoblasts have matured, these two cytokines have inhibitory effect on them. One possible explanation for these paradoxical actions is that IL1 and TNF $\alpha$  provide the osteoblasts for subsequent phase of bone formation after they have initiated the resorptive phase of bone remodeling.

The second group includes transforming growth factor beta (TGF $\beta$ ), insulin like growth factor 1 and 2 (IGF1, IGF2) and bone morphogenetic proteins (BMP). These agents stimulate both, osteoblast precursor proliferation as well as mature osteoblast function.

In a healthy steady state actions of osteoblasts and osteoclasts are kept in check by all these cytokines.

#### **Cytokine Production In RA:**

Cytokines can be classified in to three groups according to their role in inflammatory arthritis like RA.

1. Pro-inflammatory cytokines
2. Regulatory cytokines produced by T lymphocytes
3. Anti-inflammatory cytokines

Major cytokines in the first group include TNF $\alpha$ , IL1 and IL6. These are produced in large amounts by monocytes, macrophages and T lymphocytes, the cells actively involved in rheumatoid inflammation. High levels of pro-inflammatory cytokines are produced in rheumatoid synovitis (Saxne et al 1988, Miossec et al 1986). The major targets for these cytokines are blood vessels, synovium, cartilage and bones. They lead to proliferation and neo-vascularisation of the rheumatoid synovium and also lead to proliferation and hypertrophy of the synovial cells. They have been known to cause

expression of adhesion molecules and pro-inflammatory cell migration in the synovium. As explained above, these cytokines stimulate bone resorption and osteoporosis in RA.

The regulatory cytokines produced by T lymphocytes namely IL2, IL4, IL5 and  $\text{INF}\gamma$  are thought to play a balancing role in the destruction and repair activity in RA, though studies on these cytokines give contradictory results (Miossec et al 1992). More recently the concept of inhibitory cytokines (anti-inflammatory cytokines) controlling the extent of inflammatory response has been introduced (Miossec et al 1992). IL4 and GM-CSF increase monocyte differentiation which reduces production of  $\text{TNF}\alpha$ , IL1 and IL6, the pro-inflammatory cytokines. IL4 and GM-CSF are therefore anti-inflammatory cytokines. IL1 receptor antagonist (IL1 RA) is produced by monocytes and it blocks IL1 attachment to its receptor acting as a specific antagonist. It therefore acts as another anti-inflammatory cytokine and is being actively investigated for its possible therapeutic role in RA.

#### **FRACTURES IN PATIENTS WITH RHEUMATOID ARTHRITIS:**

Apart from the underlying osteoporosis, patients with RA have an increased risk of falls secondary to the functional impairment (Tinetti et al 1988). A population based study from Mayo Clinic (Hooyman et al 1984) identified 388 women who were followed up over a 25 years period (4902 person years). They showed that the relative risk of hip fractures in patients with RA was 1.5. Univariate analysis indicated that the increased risk of fractures was associated with increasing age, earlier age at diagnosis, disability, impaired ambulation, steroid use and thin stature. Obese patients and those who were using estrogen had decreased risk of fractures. In multivariate analysis only aging, impaired ambulation and thin stature were identified as independent risk factors. The type of fractures in the study were proximal femur, proximal humerus, pelvis, distal forearm and vertebrae. The relative risk for pelvic fractures was the highest ( $\text{RR}=2.56$ ). In another retrospective study of 395 patients with RA, Michel et al (1991) by multivariate analysis identified use of corticosteroid in women and prior diagnosis of osteoporosis as important risk factors. Among patients taking 5 mg or more of prednisolone, female sex strongly predicted fractures and the 5 year probability of having a fracture was 34%. Male sex, absence of osteoporosis and patients taking less than 5 mg of prednisolone were low risk groups. Insufficiency fractures occur when normal stresses are placed on weakened bones. Stress fractures of the pelvis in RA are widely reported in the

literature (Isdale et al 1993, Peh et al 1993 and Fam et al 1983). Female sex, postmenopausal status, steroid treatment and poor mobility are risk factors for these type of fractures (Isdale et al 1993). The characteristic sites of these fractures is the body of the pubis (parasymphysial) and the sacral ala (Peh et al 1993). Plain x-ray of the pelvis is often not helpful whereas Technetium 99 bone scan is the most sensitive method of detecting stress fractures (Peh et al 1993).

### **TREATMENT OF OSTEOPOROSIS IN RHEUMATOID ARTHRITIS:**

Comparatively few studies as yet are available discussing the therapeutic measures to prevent osteoporosis in RA and no studies have looked at treatment modalities to reduce rate of fractures in RA.

There could be two possible approaches to the treatment of osteoporosis in RA:

1. To control the disease activity effectively by a) pharmaceuticals and b) newer biological agents such as IL1 RA, anti TNF $\alpha$  etc.
2. Use of 'bone active' agents such as calcium and vitamin D, hormone replacement therapy (HRT) and bisphosphonates.

### **Effect Of Disease Control On Bone Mass In RA:**

In a prospective study of metacarpal bone mass measured by radiogrammetry (Kalla et al 1991), patients aged 50 or less either stopped losing or gained metacarpal bone mass when treated with slow acting anti rheumatoid drugs (SAARD). Patients aged over 50 years however continued to lose bone though the rate of loss of bone was reduced with this treatment. As SAARD are known to control disease activity this positive effect on the bone density was most probably related to suppression of the disease rather than the positive effect of SAARD on bone itself. Apart from this paper the overall experience with SAARD is not very encouraging. In a prospective study by Reid et al (1986) gold or d-penicillamine was able to suppress the disease clinically though these patients still lost 4.3% total body calcium, which was not different from patients treated with NSAIDs alone.

Methotrexate (MTX) is being widely used in the treatment of RA but its bone toxicity is not widely publicized. Experiments in rats have shown that even short term use of MTX

can diminish bone formation rates by 60% with a resultant reduction in trabecular bone volume by 27% (Friedlaender et al 1984). This finding was later confirmed by May et al (1994) who demonstrated that prolonged MTX administration in rats leads to osteopenia by suppression of osteoblastic activity and enhancement of osteoclastic recruitment. In a recent paper Bologna et al (1994) showed that after intramuscular administration, high MTX concentrations were found in the cortical and trabecular bone of RA patients. Histomorphometric analysis on two adults being treated with MTX for psoriasis and RA showed low osteoid formation and low resorption activity (Preston et al 1993). Methotrexate osteopathy is therefore an additional factor which needs to be considered in the treatment of RA. Use of newer agents such as IL1 receptor antagonist and anti-TNF $\alpha$  are being actively investigated in the treatment of RA and their effect on bone mass in RA would be interesting to note.

#### **Use Of Bone Active Agents In RA:**

Calcium alone has been shown to be ineffective in preventing loss of bone in patients with RA (Reid et al 1986a, Hall et al 1994). In postmenopausal women, estrogen therapy has been shown to preserve the spinal and femoral neck BMD in patients with RA (Hall et al 1994). In a prospective study of 200 postmenopausal women with RA, Hall et al (1994) showed that the patients receiving HRT improved spinal bone density by 2.2% as opposed to patients treated with calcium alone, who lost lumbar spine BMD by 1.1% over 2 years. Differences between the treatment groups were not significant for the femoral neck. In 21 HRT treated patients taking corticosteroids the bone mass both in lumbar spine and femoral neck was preserved over the 2 year period. In a similar double blind prospective study on 40 postmenopausal women with active RA (van der Brink et al 1993), significant improvement in the lumbar spine and femoral neck BMD was noticed in the estrogen group compared with the placebo group.

Bisphosphonates, which are strong inhibitors of bone resorption, have recently been tried in the treatment of RA. Eggelmeijer et al (1994) found a disease suppressive effect of a single infusion of pamidronate in 30 patients with active RA. The ESR and C reactive protein levels improved significantly in patients treated with 40 mg of pamidronate, though parameters of bone metabolism (serum osteocalcin and serum alkaline phosphatase) remained unchanged. The pamidronate infusions were however associated with rapid and sustained fall in urinary calcium and creatinine ratio. Bone densitometry studies on patients with RA treated with bisphosphonates are needed.

Future studies on newer agents in the treatment of RA should include bone mass measurement as one of the outcome measures. This will clarify which agents have the capacity to control the disease and also prevent bone loss in rheumatoid arthritis.

### **CONCLUSIONS:**

1. Patients with RA lose axial and peri-articular bone mass predominantly in the first year of disease.
2. The bone loss in RA is multi-factorial, but disease activity and use of oral corticosteroids in a dose of more than or equal to 5 mg. are two most important determinants.
3. Osteoporosis in RA leads to increased risk of fractures.
4. There are no proven therapies as yet available to prevent bone loss in RA but on the basis of existing studies, early and stricter control of rheumatoid activity and use of HRT in post-menopausal women with RA is recommended.
5. Future drug trials in the treatment of RA should consider including hand bone mass measurement as one of the outcome measures.

## **Legends For The Tables:**

**Table 1: Methods of bone density measurement used in studies on patients with RA.**

**Table 2: Studies on bone mass measurement in RA.**

**CS = Corticosteroids, LS = Lumbar spine, FN = Femoral neck, HAQ = Health assessment questionnaire.**

**Table 3: Studies on biochemical markers of bone metabolism.**

**RIA = Radioimmunoassay, OC = Osteocalcin, CS = Corticosteroids, OA = Osteoarthritis.**



Table 1:

METHOD*	NAA	SPA	DPA	DXA	QCT
Site	total body	radius	spine hip total body	radius spine hip total body hand	spine
Precision †	2 - 5%	1 - 2%	2 - 4%	1 - 2%	2 - 5%
Accuracy ‡	5 - 10%	5%	5 - 10%	4 - 8%	5 - 20%
Radiation dose (mrem)	2000 - 5000	5 - 10	5	1 - 3	100 - 400

\* NAA = neutron activation analysis, SPA = single photon absorptiometry, DPA = dual photon absorptiometry, DXA - dual energy X-ray absorptiometry, QCT = quantitative computed tomography

† Precision is defined as the coefficient of variation for repeated measurements.

‡ Accuracy is defined as the coefficient of variation between the result of the measurement involved and that of a reference method (for example measurement of ashed weight).

(Adapted from: Laan RFJM et al Bone mass in patients with rheumatoid arthritis Ann Rheum Dis 1992;51:826-832.)

Table 2:

REFERENCES	PATIENT CHARACTERISTICS								BONE MASS MEASUREMENT			CONCLUSIONS		
	n =		Age in Years Mean (range)		Menopausal Status PRE : POST	Disease Duration: Years	Functional Capacity	Use of oral Corticosteroids		Site and Method	Study design		Results	
	Females	Males	Females	Males				Dose mg	Duration years				% of Normal	
Reid 1982 BMJ 1982: 285:330-332	34	29	52.6	54.2	Not available	M: 7.1 F: 10.2	Mean II	31 used oral CS  6 mg	4.6 years	Total body calcium (TBC)	Cross sectional	Steroids non-users: Male: 94.7 Female: 83.2 Steroids users: Male: 88.5 Female: 84.5	Patients had lower TBC. Oral corticosteroids lead to further loss of TBC.	
Skibsted 1984 Clin Rheum 1984; 3:201-208	94	35		53	Not Available	10.7	Mean II	24 received CS  2.5 mg - 10 mg/day		SPA: Radius	Cross sectional	Steroids non-users: 86% Steroids users: 73%	Patients had lower bone mass in the distal radius. Those on CS had further reduction in bone mass.	
Verstraeten 1986 Annals RD 1986; 45:852-857	64	-	59.2	-	All post- menopausal	11.8	Most in Class II / III	28 received CS  9 mg/day	4.7 years	SPA: Distal Radius DPA: Lumbar Spine	Cross sectional	Steroids non-users: Distal radius: 84.5% Lumbar spine: 109.5% Steroids users: Distal radius: 88.2% Lumbar spine: 96%	Very contradictory results. Patients not on steroids in fact had higher spinal BMD than controls. Only those on oral CS had lower spinal BMD than controls, but this difference was because of longer disease duration rather than steroids themselves. Despite this, there was an increased incidence of fractures (16.3%) in steroid than non steroid group (3.6%).	
Sambrook 1986 Annals RD 1986; 45:950-953	84	-	56	-	20 : 64	13	I 22 II 49 III 13 IV 0	44 received CS  8 mg/day	7.5 years	DPA: Lumbar Spine Femoral Neck	Cross sectional	Steroids users: LS 90.4 FN 87.8 Steroid non-users: LS 93.1 FN 91.1  (These differences are not significant)	Female patients had reduced BMD in spine & hip compared to controls. Low dose prednisolone was not associated with increased risk of osteoporosis.	

Reid 1986 Clin Rh 1986; 3:372-378	22	23	47	13 : 9	7.0	II	Not Available	Total body calcium (TBC)	Longitudinal over 18 months	Males: 94.2% Females: 91.1%  Annual loss: 3.7%	Patients had lower TBC and they lost 3.7% calcium per annum. 11 patients with psoriatic arthritis had normal TBC and did not lose it over one year.
Reid 1986 BJR 1986; 25:253-259	41	30	51.1 (25 - 70)	13 : 28	Mean 9.1 range (1 - 35)	1.9	30 received CS  5.7 mg 4.7 years	Total body calcium (TBC)	Longitudinal Every 6 months for 18 months	Steroid non-users : 94.5  Steroid users: 89.9	Patients had lower TBC. Pts on oral CS $\leq$ 5mg per day did not lose further Calcium but those on CS > 5 mg and those not taking CS lost 2.3% & 4% Calcium/year. Low dose CS have protective role on loss of bone in RA.
Sambrook 1987 A & R 1987; 30:721-728	84	27	56 58	20 : 64	12.5	I 29 II 65 III 17 IV 0	56 received CS  9 mg/day 6.8 years	DPA: Lumbar Spine Femoral Neck	Cross sectional	No figures given but BMD at both sites significantly reduced in pts compared to controls ( $P < 0.001$ ) for both sexes.	Patients had reduced BMD in spine & hip. Low dose prednisolone (8mg) did not affect bone loss. By multiple regression analysis, steroid dose or duration did not correlate with BMD, physical activity did.
Sambrook 1988 A & R 1988; 31:973-978	49	-	61.3 -	All post-menopausal	Not Available	I 8 II 31 III 10 IV -	22 received CS  8.2 mg/day 10 years	DPA: Lumbar Spine Femoral Neck	Cross sectional	Steroids non-users: LS 97% FN 90% Steroids users: LS 93% FN 88%	Only those on oral steroids had significantly lower spinal BMD than controls. All patients had lower Estrone and Androgen levels than controls. Oral steroids lowered the sex hormone levels further.
Sambrook 1989 Annals RD 1989; 48:535-538	30	-	54 -	Not Available	12.5	I 9 II 17 III 4 IV 0	15 received CS 6.6 mg per day Duration not available.	DPA : Lumbar Spine Femoral Neck	Longitudinal Over 2 years	Lumbar spine: 89.2 Femoral neck: 85.7	Patients had significantly low BMD in spine & hip compared to controls. Low dose prednisolone (6mg/day) was not associated with increased risk of osteoporosis.

O'Malley 1989 Radiology 1989; 170:501-505	45	30	56.6 (24-78)	61.7 (41-77)	Not Available	Not Available	Not Available	Not Available	DPA DXA Lumbar Spine and Femoral Neck	Cross sectional	Female Lumbar Spine: 95 Female Femoral Neck: 80  Male Lumbar Spine: 86 Male Femoral Neck: 88	Patients had lower BMD in spine & hip. There was a good correlation between DPA & DXA results.
Sambrook 1990 A & R 1990; 33:615-622	17	-	55.4 (37-66)	-	5 : 12	1.2 (0.2 - 2.8)	Not Available	Not Available	QCT: Distal Radius  DPA: Lumbar Spine	Longitudinal over 2 years	Distal radius: 6.1% annual loss in pts with RA. No loss in controls. No change in spine BMD in pts or controls.	Rapid trabecular bone loss occurs in distal radius in pts with RA. Not so in controls. No change in LS and mid shaft radius either in pts or in controls.
Sambrook 1992 J of Rh 1992; 19:357-361	38	-	57.1	-	All post-menopausal	13.2	Median II	10 received CS 6.2 mg 11 years	DXA: Lumbar Spine Femoral Neck	Longitudinal over 4 years.	No figures given. Steroid users lost 0.5% BMD/yr from Spine. Steroid non-users and those on HRT did not lose Spinal BMD. All lost FN BMD.	All patients lost hip BMD but only those on oral steroids lost spinal BMD. Estrogen supplement protected against spinal bone loss but not against hip bone loss in patients on steroids.
Laan 1992 BJR 1992; 31:91-96	46	28	58.9 (26-86)	61.4 (32-87)	13 : 33	10.2 (1.9 - 54)	0.61	30 received CS 7 mg/day 6 years	QCT Spine	Cross sectional	No comparison with normals available but pts on CS had 34% lower BMD in spine than those without CS.	Long term oral CS in a dose of $\leq 10$ mg cause significant reduction in trabecular & cortical bone mass in pts with RA.
Laan 1993 Annals RD 1993; 52:21-26	58	39	57.5 (27-78)	57 (30-80)	17 : 41	2.6 (0.3 - 5)	0.26	21 received CS Details Not Available	DXA: Lumbar Spine and Femoral Neck	Cross sectional	Z scores: Female LS: +0.33 Female FN: -0.27 Male LS: -0.67 Male FN: -0.33	Disease duration, disease activity (ESR) & functional impairment (HAQ score), independently of each other, contribute to the bone loss of proximal femur in pts with RA.

Laan 1993 Ann Int Med 1993; 119: 963-968	28	12	54.9	-	13 : 15	2	0.63	20 received CS  7.5 mg    20 weeks	QCT: Lumbar Spine	Double blind - placebo controlled prospective	On steroids (20 weeks): lose 8% trabecular BMD 2% cortical BMD Off steroids (14 weeks): Gain back 5% trabecular BMD 1% cortical BMD  Those note on steroids do not lose bone.	Low dose corticosteroids lead to reversible bone loss in patients, mainly in the trabecular bone.
Gough 1994 Lancet 1994; 344:23-27	99	49	Not Available		28 : 71	Mean: 1	HAQ >1 82 pts <1 66 pts	85 on < 1mg/day 7 on 1 - 5mg/day 10 on > 5mg/day	DXA: Lumbar Spine Femoral Neck	Longitudinal over 3 years	No difference in spinal/hip BMD between patients and controls at entry, but over 1 year patients lost 1% in L spine, 2% in F neck. Early RA (females only) lost 2.5% in L spine & 3% in F neck.	Over 1 year the early RA pts lost higher bone mass both in LS & FN than pts with longer duration of RA. Steroid dose of 1 - 5 mg/day caused significantly loss of BMD in spine/FN. The loss of bone mass correlated with active disease (high CRP) and with worse disability (high HAQ).
Shenstone 1994 BJR 1994; 33:541-545	40	27	Pre: 36.5 Post: 65	62	16 : 24	Median: 2	0.62	None	DXA: Lumbar Spine Femoral Neck	Longitudinal	No difference in LS & FN BMD between patients and controls. Patients with disease duration <6/12 lost 3.9% FN BMD compared to 0.2% loss in the remainder of cohort.	Only patients with disease duration <6 months lost significant hip BMD. Hip BMD changes correlated with disease duration & spinal BMD changes correlated with initial Stoke index.
Peel 1994 A & R 1994 37:983-991	70	Nil	64		All post- menopausal	Range 3-45 years	Range I - III	All received CS 19 on 2.5 mg/day 50 on 5mg/day 1 on 10 mg/day	DXA: Total body Lumbar spine Femoral Neck Hand	Cross-sectional	% Decrease in BMD in patients as compared to controls Hand:            22.7 Lumbar Spine: 10.7 Femoral Neck: 16.3 Total Body:    11.3	Hand BMD measurement with DXA correlated with BMD at other sites. In early RA patients, hand BMD correlated with grip strength and inversely with ESR.
		Also Early RA: 16        4	Range 23-74		Not given	Less than 3 years	Grade I & II	1 on 5mg/day				

Table 3:

REFERENCES	PATIENT CHARACTERISTICS							MEASUREMENT OF BONE METABOLISM			CONCLUSIONS
	n = Females Males	Age	Menopausal Status	Disease Duration: Years	Functional Capacity	Use of Corticosteroids Dose                      Duration		Variable measured and Method	Study Design	Results	
Sambrook 1985 Annals RD 1985; 44:575-579	17	-	55	5 : 12 Pre    Post	1	Not Available	Not Available	S. Osteocalcin RIA	Cross sectional	Marginally reduced OC levels in patients, but not statistically significant.	No difference in serum osteocalcin levels in early RA & controls.
Weisman 1986 Arch Int Med 1986; 146:701-704	38	43	55	Not Available	14	Not Available	38 on oral CS 6 mg                      not given	S. Osteocalcin RIA Alk PO <sub>4</sub> (Total)	Cross sectional	40% & 62% reduction in OC values in male & female patients respectively. Total Alk PO <sub>4</sub> levels in female pts were higher than controls.	Patients with RA had lower levels of serum osteocalcin than controls. However, on stratification, only those on steroids had significantly lower levels than controls. Steroid non-users had similar levels to the controls.
Ekenstam 1986 Annals RD 1986; 45:485-490	26	10	50	Not Available	Not Available	Not Available	9 received 20 mg for 1 week	S. Osteocalcin RIA	Cross sectional	Reduction of 54% in females & 22% in male patients in OC values as compared to controls. 1 week of oral CS further reduced OC levels significantly.	Reduced levels of osteocalcin in inflammatory arthritis suggest reduced bone turnover. Oral CS suppresses bone turnover further.
Verstraeten 1986 Annals RD 1986; 45:852-857	64	-	59.2	All post- menopausal	11.8	Most in Class III/IV	28 on oral CS 9 mg                      4.7 years	Alk PO <sub>4</sub> (Total)	Cross sectional Controlled	Marginally raised total Alk Phosphatase in patients with RA. No effect of oral CS on the Alk PO <sub>4</sub> levels.	This study mainly looks at the changes in LS & distal radius bone mass. The biochemical data is insufficient and not impressive.



Robins 1986 Annals RD 1986; 45: 969-973	Total 11 (RA) (details not available) Total 20 (OA)		RA 63 OA 65	Not Available	Not Available	Not Available	Not Available	Urinary pyridinoline (PYD) by ELISA (Urine collection <u>not</u> early morning)	Cross sectional Controlled	422% increase in excretion of PYD in patients with RA, & 104% increase in patients with OA.	Patients with RA & OA excrete larger amounts of PYD than controls. In this study the urine was not collected in any uniform way & no information is given about use of steroids, menopausal status, etc.
Seibel 1989 J of Rh 1989; 16:964-970	36	5	61	Not Available	10	I 6 II 13 III 20	9 on oral CS 4.8 mg 3 years	Urinary Pyridinoline (PYD or HLP) & deoxypyridinoline (DPD or LP) by HPLC.	Cross sectional Controlled	200% increase in PYD & 205% increase in DPD in patients with RA. Active RA had 100% higher PYD levels than inactive RA.	Both PYD & DPD excretion in urine is significantly raised in RA, but PYD excretion, which is a marker of cartilage & bone degradation, corre- lates with disease activity.
Magaro 1989 BJR 1989; 28:207-211	42	-	52	Not Available	<5 years : 21 >5 years : 21	I - II 20 III - V 22	None	S. Osteocalcin RIA	Cross sectional Controlled	Patients with active RA had 82% higher OC than controls & patients with inactive RA. (Activity defined by ARA criteria.)	OC levels depend on activity of RA. Those with high activity had higher OC levels. Alk PO <sub>4</sub> correlated with OC levels only in active group.
Black 1989 Annals RD 1989; 48:641-644	Details Not Available Total 19		53	Not Available	Not Available	Not Available	None	Urinary Pyridinoline (PYD or HLP) & deoxypyridinoline (PDP or LP) by HPLC.	Cross sectional Case control	311% increase in PYD & 38% (not significant) increase in DPD in patients with RA. PYD levels correlated with CRP, ESR and negatively with grip strength.	PYD excretion in urine is significantly raised in patients with RA & it correlates with disease activity & inversely with function.
Pietschmann 1989 Annals RD 1989; 48:654-657	21	8	53	Not Available	8	II 22 III 7	None during the study, though 20 had received CS in varying doses in the past.	S. Osteocalcin RIA	Cross Sectional Controlled	No significant difference between patients & controls, those who took CS & those who did not.	Normal osteocalcin levels in patients with RA suggest normal rate of bone formation! Osteo- penia in RA therefore could be secondary to increased bone resorp- tion rather than reduced bone formation.

Marthoffer 1991 J of Rh 1991; 18:1158-1162	55	17	59.8	Not Available	6.86	I - II 15 III - IV 40 Not available on 17	Details not available but 34 pts received <10 mgs/day	S. Osteocalcin RIA	Longitudinal Controlled	No difference in OC values in controls & patients, either early or advanced RA. However, OC levels were 58.5% higher in patients with late onset (>65 years) RA, but reduced over 1 year.	Patients with late onset RA had more active disease (↑↑ ESR) & high levels of OC. Over one year the ESR & OC both dropped. OC therefore depends on disease activity & <u>not</u> on the onset. OC correlates with ESR ( $r=0.64$ , $p=0.007$ ) but not with Alk $PO_4$ .
Gough 1994 Annals RD 1994; 53:14-17	42	20	55	14 : 28 Pre Post	1 year	HAQ 1	8 took oral CS No details given.	Urinary Pyridinoline (PYD or HLP) & deoxypyridinoline (DPD or LP) by HPLC, and spine & femoral neck BMD by DXA.	Longitudinal Controlled	66% increase in PYD & 88% increase in DPD in patients when compared to controls. Patients with active RA (CRP >20) lost 2% & 3.5% BMD in spine & hip when compared to 1% gain in both sites in patients with inactive disease (CRP <20).	All patients with RA have higher levels of PYD & DPD in the urine. Patients with active RA lose more bone mass in the spine & hip, and have higher PYD excretion. The loss at femoral neck correlates with PYD excretion.



**DEVELOPMENT AND VALIDATION OF THE METHOD OF MEASURING HAND BONE  
MINERAL CONTENT BY DUAL ENERGY X-RAY ABSORPTIOMETRY**

## **INTRODUCTION**

This chapter describes the development of the technique to measure hand bone mineral content by dual energy x-ray absorptiometry (Hologic QDR 1000 machine). This machine is optimally designed to measure the BMC in hip and spine with an accuracy of 2%. However measurement of BMC by the standard Hologic lumbar spine programme is both dependent on the soft tissue thickness and is non-linear for variations in BMC at the lower range as found in the hand.

To overcome this problem, Pye and Law (1990) described a method of scanning the hand on a built up plate made of perspex-aluminium to shift the overall hand BMC and the soft tissue found around the hand, towards the range observed in scanning the lumbar spine of normal subjects. The plate "hardens" the X-ray beam by removing the lower energy X-rays at the time of scanning.

We decided to use such a plate for our initial experiments in one of the three protocols we developed to measure hand BMC using Hologic QDR 1000 machine. We have compared the reproducibility and accuracy of these three methods using two human hand specimen. In this chapter we also describe more fully the method applied during the rest of the studies.

## **AIMS**

The aim of this study was to develop a method of measuring hand bone mineral content (BMC) that could be applicable to patients with rheumatoid arthritis. This method needs to be objective, reproducible and accurate and considering the progressive nature of RA, quick and comfortable to the patient. It should also be not affected by hand deformities.

## **MATERIALS AND METHODS**

### **Development Of The New Method**

Bone mineral content of the hand was measured by DXA using a Hologic QDR 1000 machine. To determine the optimum hardening of the x-ray beam, successive layers of aluminium were added on a 2 centimeter perspex plate until the values of the Hologic parameters  $k$ , which is a function of the tissue attenuation characteristics of the beam, and  $d$ -theta which is used to convert beam attenuation reading to BMC values, matched those obtained by scanning the Hologic spine phantom. The plate was made large enough to fill the entire hand area.

The bone to soft tissue threshold, which differentiates bone from the soft tissue when analyzing, was altered to threshold 8, 8, 8 after testing several values on a number of hand scans. This threshold gave the best compromise between a reasonable definition of the bone outline without losing the small bones in the terminal phalanges. The bone edge detection between the metacarpals needed operator intervention to paint out the first interosseous muscle, whose comparative bulk can contribute up to 5% of the apparent bone mass. The other interossei were left untouched to minimize the operator induced variability.

### **Evaluation Of Linearity**

The use of the aluminium-perspex plate had no effect on the non-linearity of the Hologic spine analysis programme for variations in BMC at lower range. To achieve linearity, an aluminium step wedge resembling bone thickness' of the hand was prepared to simulate hand and was scanned on this plate. Proportionality between the derived BMC result and the varying thickness' of this plate was achieved by changing the Hologic linearity coefficients Q1, Q2 and Q3.

The above parameters were included in the analysis software and invoked automatically when scanning hands.

## **Study Subjects**

We selected 9 healthy normal volunteers (3 males, 6 females, age 26-53) to participate in the initial studies on measurement of hand bone mineral content by DXA. All these volunteers were hospital employees and did not suffer from any condition affecting hand bones. They had never used oral corticosteroids and none of the female volunteers were pregnant. All study subjects gave informed consent and the study was approved by the Ethical Committee of the Royal Cornwall Hospital, Truro.

## **Evaluation Of Reproducibility**

The reproducibility of the method was determined by scanning 10 hands of normal volunteers. Each hand was positioned palm down on the plate with fingers extended, and scanned 5 times with repositioning between measurements.

## **Effect Of Hand Position**

Five normal volunteers had their hands scanned in two different attitudes, flat on the plate and with slightly flexed fingers simulating a rheumatoid hand, to assess the effect of position on the measurement of hand BMC and hand bone mineral density (BMD) (Figures 1 and 2). In two volunteers, larger changes in hand positioning were studied by scanning one hand flat on the plate and also after making a tight fist.

## **Evaluation Of Inter-Observer Variability**

The inter-observer variability of the hand BMC analysis was assessed by two observers analyzing three hand scans each five times.

## **Evaluation Of Alternative Protocols For Measuring Hand BMC And Assessment Of Accuracy**

The accuracy experiments were conducted on two pathology hand specimens, one 'normal' and one 'osteoporotic', acquired from the Pathology Department of the Royal Cornwall Hospital, Truro.

The first 'normal' hand was from a 56 year old male patient with epilepsy who underwent an amputation for acute pressure gangrene of the left upper limb. He did not suffer from

arthritis and did not have any long-term illness affecting the left hand. The x-ray of the amputated hand revealed bones with no radiographic evidence of osteoporosis.

The second 'osteoporotic' hand specimen was from a 62 year old patient with malignant mesothelioma. The intractable pain and swelling in the left upper limb led to the palliative amputation of his left upper limb. He had not used his left upper limb for over six months and the x-ray of this hand before amputation revealed osteopenic bones. After amputation, both patients gave consent for their hands to be used for research purposes.

Each hand was scanned twenty times each using following three protocols:

Protocol 1: Hand positioned on the custom-built plate, image acquired by Hologic lumbar spine software and analyzed using the method described above.

Protocol 2: Hand positioned on scan table without using the plate, image acquired by Hologic lumbar spine software and analyzed by Hologic forearm software.

Protocol 3: Hand positioned on scan table without using the plate, image acquired and analyzed by Hologic forearm software.

To determine the accuracy of each method, the results of the observed hand BMC were compared with the ashed weight of hand bones.

### **Ashing Protocol**

Each hand was dissected and individual bones were separated from the soft tissue. The bones were 'fixed' in formalin overnight before drying in a small oven for two hours at 80° C. They were then sawn into small sections, each approximately 1cm<sup>3</sup> to fit in the crucibles used for ashing. A set of clean, dry crucibles was weighed and the bone sections distributed amongst them so that there were approximately two sections per crucible. These were then baked at 700° C for two hours and allowed to cool. A solution of 4M nitric acid was pipetted over each sample and the crucibles were returned to the oven and the temperature slowly increased to 700° C. The crucibles were then baked for a further one hour. After incineration was complete, the crucibles were allowed to cool and were weighed. The final ash weight was then calculated and totaled over all crucibles.

## **RESULTS**

### **Development Of The Perspex-Aluminium Plate And Linearity**

For our scanner, a plate thickness of 7 mm aluminium plus 20 mm perspex was found to give closest match to the value of k and d-theta used in the Hologic spinal analysis programme. We therefore prepared a custom-built plate of this thickness for further use in hand BMC measurements. To obtain linearity between the results of BMC of small thickness hand bones, Hologic linearity coefficients were altered to Q1=0.203, 0.226, Q2=0.405, 0.427 and Q3=0.608, 0.616, which were used in all further analysis.

### **Experiments On Volunteers: Reproducibility, Effect Of Hand Position And Inter-Observer Variability.**

For the method of acquisition and analysis described by us, the coefficient of variation (CV) for hand BMC was 2.3% and for hand BMD 1.3% (Table 1).

Table 1: Hand Bone Mineral Content Reproducibility:

	A	B	C	D	E	F	G	H	I	J
<b>BMC: 1</b>	38.2	35.14	29.39	29.81	32.56	35.1	30.91	29.68	32.22	33.04
<b>BMC: 2</b>	37.44	35.74	29.41	30.68	33.1	35.5	30.28	30.01	32.46	33.3
<b>BMC: 3</b>	37.92	36.1	29.49	30.42	32.96	35.3	31.34	30.83	33.08	32.84
<b>BMC: 4</b>	38.54	36.2	30.23	31.4	33.4	36.4	31.3	30.43	33.1	33.38
<b>BMC: 5</b>	37.9	35.83	30.15	29.54	32.94	36.38	30.7	30.79	32.85	32.98
<b>MEAN</b>	38	35.8	29.73	30.37	32.99	35.73	30.9	30.34	32.74	33.1
<b>STD</b>	0.363	0.371	0.374	0.657	0.271	0.548	0.394	0.446	0.348	0.201

Note: Ten hands (A to J) were scanned five times each. STD = Standard Deviation.

Changing the hand position from flat to slightly flexed (simulating a rheumatoid hand) or tight fist did not alter the precision of the BMC result, though it did alter the BMD, which is area dependent. The hand BMD increased by 13.1% (Figures 1 and 2) with the hand in a slightly flexed position and by 30.8% in a tight fist position (Table 2). The first inter-osseous muscle could not be painted out with the hand in a tight fist position. Despite this, the hand BMC did not change with the hand in flat or a fist position (Figures 3 & 4).

Table 2: Effect of hand position on hand BMC: Five hands scanned flat and semiflexed.

POSITION	FLAT		SEMI-FLEXED	
	BMC	BMD	BMC	BMD
HAND 1	35.14	0.387	35.88	0.426
HAND 2	31.07	0.344	31.82	0.456
HAND 3	46.70	0.403	46.17	0.454
HAND 4	31.22	0.321	31.81	0.432
HAND 5	37.49	0.375	37.68	0.428

Analysis by a second observer did not change the precision of BMC or BMD result (see Table 3).

Table 3: Inter-Observer Variability

	OBSERVER:1					MEAN	STD		OBSERVER: 2					MEAN	STD
Scan 1	37.44	37.42	37.48	37.42	37.4	37.43	.027	37.39	37.43	37.44	37.42	37.45	37.42	.02	
Scan 2	29.16	29.15	29.22	29.17	29.14	29.16	.025	29.21	29.2	29.16	29.18	29.16	29.18	.02	
Scan 3	43.28	43.29	43.26	43.23	43.29	43.27	.011	43.22	43.24	43.20	43.26	43.30	43.24	.02	



## Evaluation Of Alternative Protocols For Measuring Hand BMC And Assessment Of Accuracy

For each of the three protocols, the observed hand BMC, coefficient of variation and scanning times as well as the final ashed weights of both hands are shown in Table 4.

Table 4: Three Protocols Of Hand BMC Measurement

	Protocol 1		Protocol 2		Protocol 3		Ash Weight
	BMC	CV	BMC	CV	BMC	CV	
Normal Hand	35.47	1.18	24.76	0.97	26.48	2.91	27.48
Osteoporotic Hand	32.43	1.11	14.54	4.26	19.38	3.3	23.6
Ratio:	1.09		1.70		1.36		1.16
Normal/Osteopenic							
Scan Time	10 Minutes		10 Minutes		20 Minutes		
Analysis Time	5 Minutes		8 Minutes		8 Minutes		

Note: The BMC and ash weights are in grams.

Protocol 3 was the slowest, requiring twice the scanning time as compared to protocols 1 and 2. For the first hand, protocols 2 and 3 were more accurate with better reproducibility than protocol 1, but with the osteoporotic hand, protocols 2 and 3 failed to delineate the bones properly and lost the advantage of superior accuracy. This is reflected in the ratio of normal to osteoporotic hand BMC. Protocol 1 effectively delineated normal as well as osteoporotic bones and thus the ratio of normal to osteopenic hand BMC by protocol 1 was the closest to that by ashing. It however over-estimated the amount of hand BMC by an average multiplicative factor of 1.3.

## **COMMENTS AND CONCLUSIONS**

1. We have developed a novel method of scanning and analyzing hand BMC using dual energy x-ray absorptiometry by altering an existent spine protocol.
2. Our method is reproducible and accurate for both, normal and osteoporotic hands.
3. In normal volunteers, the reproducibility of our method did not depend on the observer.
4. Change in hand position from flat to semi-flexed (simulating a rheumatoid hand) did not change the BMC significantly, but increased the BMD which is dependent on the surface area. For long term studies therefore hand BMC, which is area independent, is a preferred measurement.
5. The other two methods, using existent forearm protocols, are either less accurate than our method when dealing with an osteoporotic hand, or require much longer scanning time. This may prove problematic in dealing with patients with painful arthritis affecting hands.
6. We therefore used Protocol 1 for the measurement of hand bone density in further experiments described in this thesis.

Figure 2: The same hand scanned in a semi-fixed position. Note the effect on hand BMC and hand BMD.

Figure 1: Hand scan of a normal volunteer using perspex-aluminium plate. Hand position: flat.



Figure 2: The same hand scanned in a semi-flexed position. Note the effect on hand BMC and hand BMD.

Figure 2: Hand position BIL



Figure 4: Hand position: flat.

Figure 3: Hand position: flat.

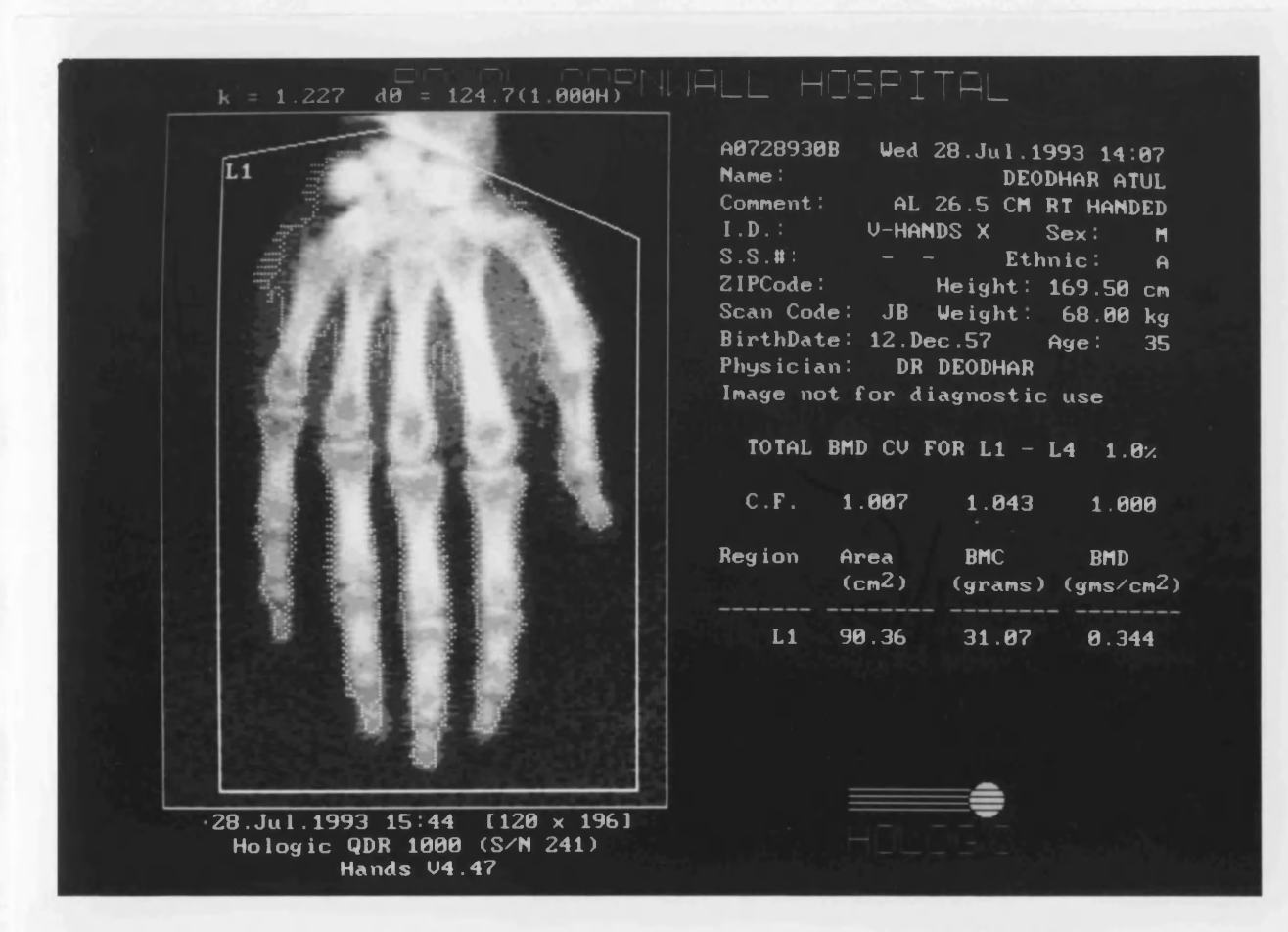
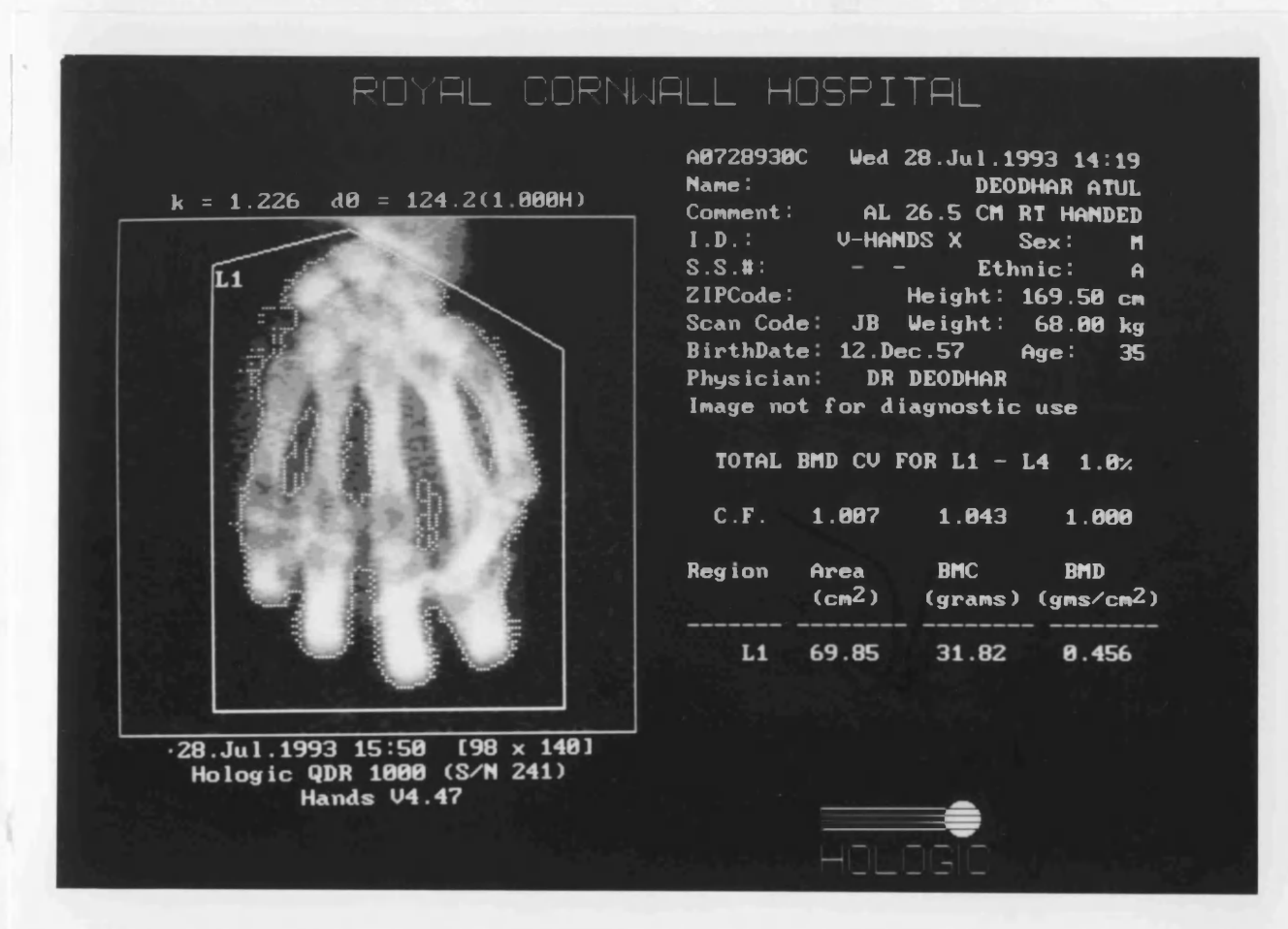


Figure 4: Hand position: fist.



**MEASUREMENT OF HAND BONE MINERAL CONTENT BY DUAL ENERGY X-RAY  
ABSORPTIOMETRY IN NORMAL VOLUNTEERS**

## **INTRODUCTION**

Measurement of hand bone mineral content (BMC) by dual energy x-ray absorptiometry (DXA) is an accurate and reproducible technique as shown in the last chapter. It does not depend on the hand position or on the operator. This chapter describes the application of this technique to normal volunteers to estimate the normal ranges of hand BMC in males and females and also to assess the effect of sex, body size and hand dominance on hand BMC in healthy individuals.

## **SUBJECTS AND METHODS**

Ninety five normal volunteers (46 males, age 24 to 81, median 33 and 49 females, age 20 to 83, median 41, 31 pre- and 18 postmenopausal) were enrolled from the hospital staff, spouses and voluntary workers in the Royal Cornwall Hospital, Truro. Written informed consent was obtained. There were no major differences in the use of the hand and none of them were manual labourers. Those suffering with any disease affecting the hands, those who had used steroids any time in their life and in case of women, those who were pregnant or were likely to be pregnant were excluded.

Variables recorded for the healthy volunteers were age, weight, height, forearm span (length of forearm from the ulnar styloid to the olecranon process measured by a specially made measuring bench on which the forearm was positioned), hand volume (measured as water displaced by hand distal to the ulnar styloid process, the reproducibility of this method was  $CV = 1.8\%$ ), hand dominance and the position of the hand while scanning. The physical characteristics of the volunteers are shown in Table 1. All volunteers underwent hand BMC measurements on both hands by DXA.

Thirty seven out of ninety five volunteers (9 males and 28 females, 14 pre and 14 postmenopausal) agreed to be re-scanned and underwent a repeat measurement of hand BMC after 1 to 4 years to establish the annual rate of change in hand BMC in healthy controls.



**Table 1: Physical characteristics of normal volunteers. Median (IQR).**

	<b>Male</b>	<b>Female</b>
	<b>n=46</b>	<b>n=49</b>
<b>Age in years</b>	<b>42 (17)</b>	<b>43.7 (18.3)</b>
<b>Age range</b>	<b>24-81</b>	<b>20-83</b>
<b>Premenopausal</b>	<b>--</b>	<b>23</b>
<b>Weight in kg</b>	<b>75.4 (9.7)</b>	<b>63.8 (10)</b>
<b>Height in cm</b>	<b>175.4 (6)</b>	<b>161.1 (6.8)</b>
<b>Dominant hand volume in cc</b>	<b>345 (181)</b>	<b>313 (43.1)</b>
<b>Forearm span in cm</b>	<b>27 (4.2)</b>	<b>24.3 (1.6)</b>

## **ANALYSIS**

The statistical analysis was carried out using the NCSS statistical package. For comparisons within the group, paired t-test and Wilcoxon matched pair signed ranks test were used depending upon the distribution of the variables. Intergroup comparisons were performed by Mann-Whitney U test or unpaired T-test according to distribution of variables. Spearman's correlations were calculated. Partial correlations were calculated to allow for the effects of covariates such as age, height and weight. Differences between groups were corrected for other covariates by using multiple regression.

## **RESULTS**

### **Hand BMC at Baseline**

The hand bone mineral content in male and female volunteers at baseline is shown in Figure 1 and Table 2.

Table 2: Dominant and non-dominant hand BMC in normal volunteers.

	Dominant Hand			Non-Dominant Hand		
	Median	IQR	Range	Median	IQR	Range
<b>Male</b>	46.71	7.48	32.56, 62.98	45.7	7.47	31.59, 61.63
<b>Female</b>	31.35	6.17	19.11, 39.79	31.2	5.85	19.28, 37.66

In males the mean total hand BMC (dominant plus non-dominant) was 90.9 gms (95% CI 86.9 - 95, SD 13.7). In females the mean total hand BMC was 62.2 gms (95% CI 59.8 - 64.5, SD 8.3).

### Effect Of Body Size On Hand BMC

The effect of the covariates age, height, weight, forearm span and hand volume on the hand BMC was examined by Spearman's correlation. In males, the total hand BMC correlated with forearm span ( $r=0.5$ ,  $p=0.0006$ ), height ( $r=0.57$ ,  $p<0.0001$ ), weight ( $r=0.58$ ,  $p<0.0001$ ) and hand volume ( $r=0.66$ ,  $p<0.0001$ ). In females the total hand BMC correlated with forearm span ( $r=0.3$ ,  $p=0.03$ ), weight ( $r=0.4$ ,  $p=0.003$ ), hand volume ( $r=0.49$ ,  $p=0.0008$ ) and height ( $r=0.66$ ,  $p<0.0001$ ). No significant correlation was found between hand BMC and age in males and females (Figure 2).

### Effect Of Hand Dominance On Hand BMC

The hand volume was significantly greater in dominant than non-dominant hands of males ( $p = 0.0001$ ) and females ( $p = 0.005$ ), assessed by Wilcoxon matched pairs signed ranks test. In spite of this difference in the hand volumes, there was no statistical difference between the BMC of the dominant and non-dominant hands in male and female volunteers as shown by paired t-test.

### Comparison Of Hand BMC In Male And Female Volunteers

After correcting for height, weight, volume and forearm span, male volunteers had significantly more bone mass than female volunteers ( Figure 1,  $p= 0.01$ ).

### Hand BMC At 12 Months

The comparison of the hand BMC results at baseline and at 12 months are shown in Table 3. Over 12 months, both male and female normal controls did not have any significant change in their hand BMC. When the female control population was divided according to their menopausal status, the postmenopausal group was found to lose significant amount of hand BMC over one year ( $p=0.001$ ).

Table 3: Percent Change In Dominant Hand BMC In Normal Volunteers Over One Year.

	n=	Percent change	IQR	p=
<b>Males</b>	9	0.9	1.73	0.2
<b>Females (Pre-M)</b>	14	-0.17	3.5	0.6
<b>Females (Post-M)</b>	14	-1.6	3.11	0.001
<b>Females (all)</b>	28	-0.9	1.92	0.08

### COMMENTS AND CONCLUSIONS

1. In normal volunteers, the hand BMC was dependent on the body size and correlated with hand volume, weight and height.
2. Unlike the BMC in spine and hip, there was no significant correlation between hand BMC and age in volunteers.
3. In the population studied, there was no significant difference between the hand BMC of the dominant and non-dominant hand either in males or in females
4. After correcting for body size, males had higher hand BMC than females.
5. On annual follow-up, male and pre-menopausal female volunteers did not lose any hand BMC, but post-menopausal females did lose significant amount of hand BMC

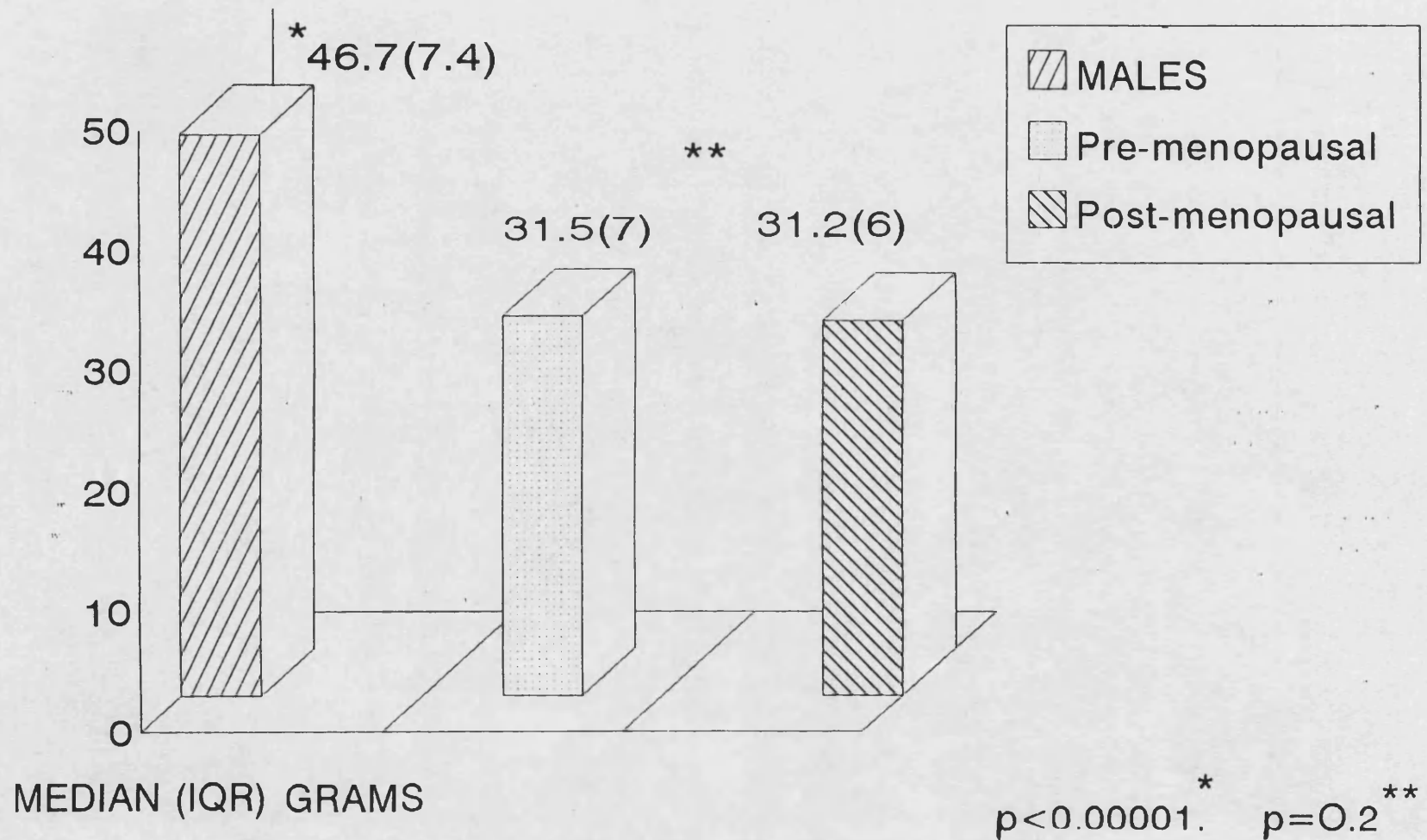
## List of Figures

Figure 1: Dominant hand BMC in grams in normal volunteers. After correcting for body size, male volunteers had significantly higher BMC than female volunteers ( $p=0.01$ ).

Figure 2: Hand BMC plotted against age in male and female volunteers.

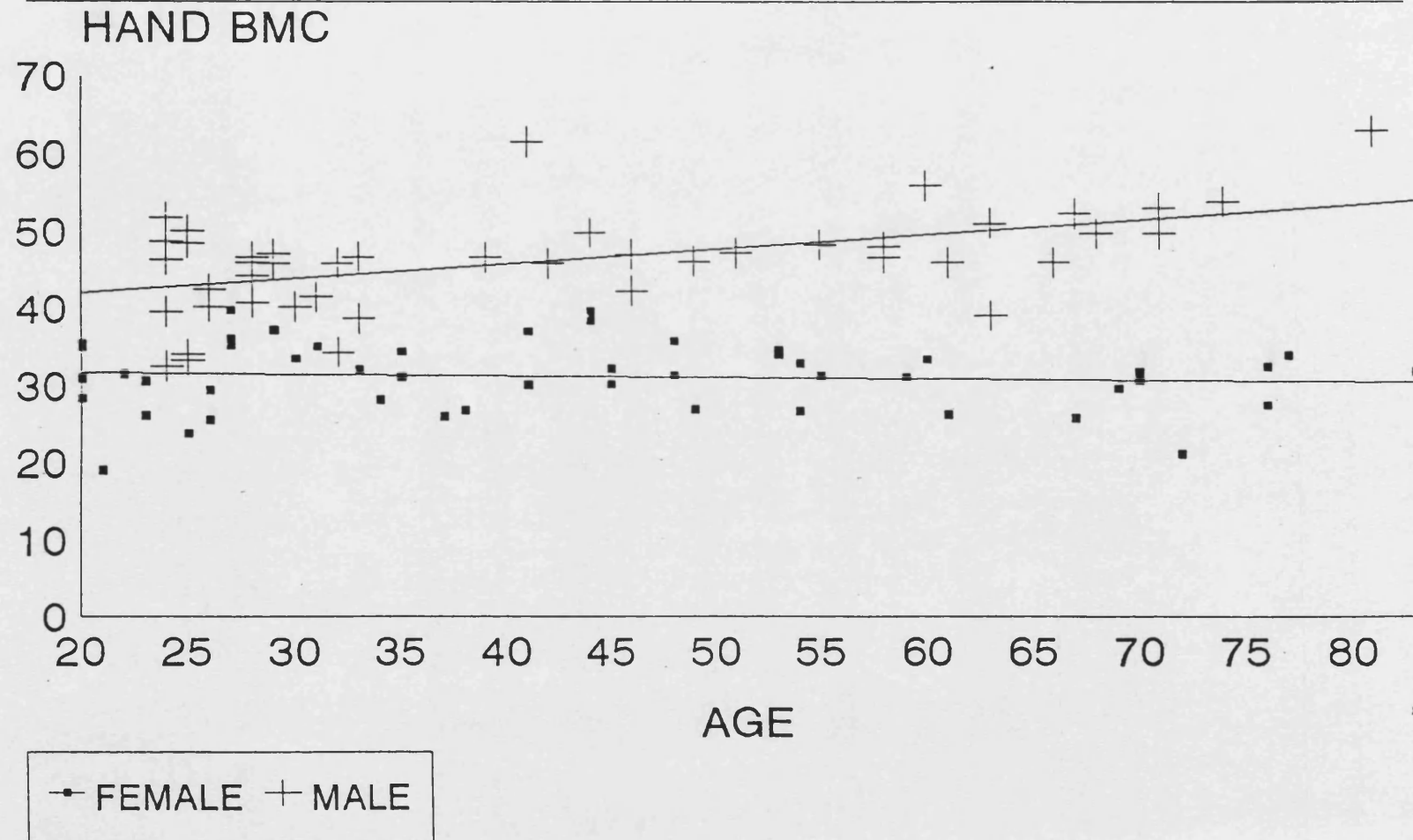
# HAND BONE MINERAL CONTENT

NORMAL VOLUNTEERS, DOMINANT HAND



# HAND BONE MINERAL CONTENT

NORMAL VOLUNTEERS: MALE AND FEMALE



**MEASUREMENT OF HAND BONE MINERAL CONTENT BY DUAL ENERGY X-RAY  
ABSORPTIOMETRY IN PATIENTS WITH ESTABLISHED RHEUMATOID ARTHRITIS: A  
CROSS-SECTIONAL AND ONE YEAR LONGITUDINAL STUDY**

## **INTRODUCTION:**

Periarticular as well as generalized osteoporosis are some of the commonest extraarticular manifestations of rheumatoid arthritis (Woolf 1991). Many studies have looked at generalized osteoporosis in patients with RA by measuring the bone mineral density in spine and hip; and measurement of BMD at distal radius has been used to assess the periarticular osteoporosis (for details, see Table 2, Chapter 2). Our technique of hand bone densitometry may provide a new tool to study both, generalized and periarticular osteoporosis in patients with RA. We have therefore applied it to a cohort of patients with RA in a cross-sectional study and then in a one year longitudinal study to see whether bone density is reduced in rheumatoid arthritis, whether there is an ongoing loss and whether the bone density or the bone loss correlate with other measures of disease activity and outcome.

## **SUBJECTS AND METHODS: THE CROSS-SECTIONAL STUDY**

Fifty-six patients (22 males and 34 females) with rheumatoid arthritis of differing severity and with duration greater than two years had both hands scanned to determine BMC. These patients were chosen at random from out-patients and in-patients of the Duke of Cornwall Rheumatology Unit, Truro. The clinical activity was assessed by Ritchie Index (Ritchie et al 1968) and a swollen joint score (number of joints with tenderness and synovitis), the functional status by Health Assessment Questionnaire (HAQ) expressed as functional disability index (Fries et al 1980) and radiological severity was determined on hand X-rays by Larsen's grades (Larsen et al 1977) and Modified Sharp's method (Sharp et al 1985). The disease activity was measured by plasma viscosity and C-reactive protein. All patients underwent BMC measurement of both hands (Figure 1).

## **SUBJECTS AND METHODS: THE ONE YEAR LONGITUDINAL STUDY**

Out of the original fifty-six patients, thirty-nine (16 males aged 30-84, median 65.5 years, and 23 females aged 33-84, median 63 years, 7 pre-menopausal) attended follow up one year later. They underwent a full assessment as explained above including a repeat hand BMC measurement.



All except three patients were on DMARDs during the study period (13 penicillamine, 9 sulfasalazine, 4 sodium aurothiomalate intra-muscular, 4 methotrexate, 4 azathioprine, 1 hydroxychloroquine and 1 patient received sodium aurothiomalate injection and sulfasalazine together). During the study period none of the patients received oral corticosteroids though eleven patients had used oral steroids in the past for other reasons.

The hand BMC data on the normal volunteers (control population) described in the last chapter was used for comparison with the patients.

## **ANALYSIS**

Number Cruncher Statistical System (NCSS) statistical programme was used for statistical analysis. Hand BMC of controls and patients was compared by Mann-Whitney test. Baseline results in patients were compared with their results at month 12 by Wilcoxon matched pairs test. The differences in hand BMC (Initial - Final) were correlated with the baseline parameters of disease activity by Spearman's correlation. Comparisons between controls and patients with RA in pairs were made by carrying out an analysis of covariance with age, weight and height as covariates. Only those covariates which indicated a significant association with BMC were included in the final analysis.

## **RESULTS**

### **Results At Baseline: The cross-sectional study.**

Demographic characteristics of patients with rheumatoid arthritis are described in Table 1 and the disease characteristics of patients at the start of the study are shown in Table 2.

The corrected mean dominant hand BMC in male patients was 7.5% reduced ( $p=0.003$ ) when compared to male controls and in female patients the corrected mean dominant hand BMC was 7.8% reduced ( $p=0.01$ ) when compared to female controls (Table 3). There was no statistical difference between the dominant and non-dominant hand BMC in male and female patients with RA by the paired t-test. The hand BMC inversely correlated with age in female ( $r=-0.44$ ,  $p=0.01$ ) but not in male patients.

**Table 1: Demographic Characteristics Of The Patients:**

	<b>Male (n=22)</b>	<b>Female (n=34)</b>
<b>Median Age (Range)</b>	64 (29-83)	64 (25-86)
<b>Premenopausal</b>	--	5
<b>Median Disease Duration</b>	7 years	10 years
<b>Disease Duration Range</b>	2-25 years	2-40 years
<b>Number on DMARDs</b>	16	26
<b>Past Corticosteroid Use</b>	4	7

**Table 2: Disease Characteristics Of The Patients (n=56): The Cross-sectional Study**

	<b>Median</b>	<b>IQR</b>
<b>Early Morning Stiffness</b>	30	77
<b>Ritchie Articular Index</b>	10	14
<b>Number of Swollen Joints</b>	3	4
<b>HAQ Score</b>	1.35	1.05
<b>C Reactive Protein</b>	14.7	46
<b>Plasma Viscosity</b>	1.8	0.29
<b>Larsen Score</b>	20.5	27

**Table 3: Hand bone mineral content in patients: The Cross-sectional Study**

	<b>Control</b>		<b>Patients</b>	
	<b>Median</b>	<b>95% CI</b>	<b>Median</b>	<b>95% CI</b>
<b>Male</b>	46.53	44.7-47.9	42.70	41-44.4
<b>Female</b>	30.82	29.6-32	28.4	27.2-29.5

The Mann Whitney test was used to compare age, height and weight in volunteers and patients since the groups were not matched for these covariates. Age was significantly higher in patients when compared with volunteers (males  $p=0.0001$ , females  $p<0.0001$ ), height was lower in male patients than male volunteers ( $p=0.01$ ) but weight was not significantly different in either sexes in these two groups. The total hand BMC (Dominant and Non-dominant) in patients was therefore corrected for height and age in male patients and for age in female patients by using multiple regression with group and the covariates as independent variables. Male patients (corrected for age and height) had lower hand BMC than male volunteers ( $p=0.005$ ) and female patients (corrected for age) had significantly lower hand BMC than female volunteers ( $p<0.005$ ).

7 female and 4 male patients had used oral corticosteroids at some stage of their disease but there were no statistically significant differences in hand BMC between users and non-users of either sex using Mann Whitney test. To allow for the confounding effect of other variables, partial correlations (Spearman's) of patients total hand BMC were used eliminating the effect of age, height and weight. The hand BMC showed inverse correlation with disease duration ( $r=-0.62$ ,  $p=0.0003$ ), Larsen score ( $r=-0.62$ ,  $p=0.0002$ ) and Sharp's score ( $r=-0.69$ ,  $p<0.0001$ ) in females. In male patients the hand BMC also showed an inverse but much weaker and not significant correlation with disease duration ( $r=-0.23$ ,  $p=ns$ ), Larsen score ( $r=-0.24$ ,  $p=ns$ ) and Sharp's score ( $r=-0.31$ ,  $p=ns$ ). Figure 2 shows the correlations between hand BMC (all patients, male and female) with HAQ score, disease duration, Sharp's score and Larsen score. Ritchie index, swollen joint score, EMS or HAQ did not show any significant correlation with the hand BMC.

A comparison of hand BMC values in normal volunteers and patients with RA is shown in figure 3.

## **RESULTS AT 12 MONTHS: THE ONE YEAR LONGITUDINAL STUDY**

Thirty-nine patients (16 males aged 30-84, median 65.5 years, and 23 females aged 33-84, median 63 years, 7 pre-menopausal) participated in the longitudinal study and attended follow up one year later. They underwent a full clinical and laboratory assessment including hand BMC measurement. Over the study period, there was no significant change in the measured parameters of disease activity (Table 3) although the Larsen score and the HAQ score worsened significantly.

**Table 3: Disease Characteristics Of The Patients (n=39) At Entry And At 1 Year: The Longitudinal Study**

<b>Median (IQR)</b>	<b>Entry</b>	<b>1 Year</b>	<b>*p Value</b>
<b>EMS</b>	30(90)	30(88)	= 0.2
<b>Ritchie</b>	10(14)	10(13)	= 0.6
<b>Swollen Jts.</b>	3(3)	3(6)	= 0.5
<b>HAQ Score</b>	1.5(1.3)	1.6(1.3)	= 0.01
<b>CRP</b>	14(43)	14(44)	= 0.2
<b>P. Viscosity</b>	1.78(.26)	1.66(.16)	= 0.1
<b>Larsen Score</b>	25(30)	29(37)	= 0.001
<b>Hand BMC (Grams)</b>	30(16)	31(15)	= 0.17

Note: \*Wilcoxon matched pairs test. IQR = Inter quartile range, Ritchie = Ritchie articular index, the Larsen's score was derived from both hand x-rays.

There was a trend showing loss of hand BMC over one year in males but these changes did not reach statistical significance (Median loss 0.94 grams, 2.24%, 95% CI for percent loss -0.86 to 4.14, p=0.1) and there was no significant change in females (0.03 grams, 0.11%, 95% CI for percent loss -4.52 to 5.14, p=0.2, Table 4, Figure 2).

**Table 4: Percent Change In Dominant Hand BMC In Patients With RA Over One Year**

	<b>n=</b>	<b>Median</b>	<b>IQR</b>	<b>*p=</b>
<b>Males</b>	16	-2.24	4.33	0.1
<b>Females</b>	23	0.11	11.56	0.2

Note: \*p value refers to the paired differences between hand BMC at entry and at one year by Wilcoxon Matched Pairs Test.

The individual rate of change however varied between 8% gain to 33% loss with greatest losses in certain postmenopausal women (Figure 4). There was no correlation between change in hand BMC with parameters of disease activity.

Numbers were too small to examine the effect of past use of corticosteroids on hand BMC.

### **Effect Of Menopause On Hand BMC**

To investigate whether menopausal status affected the hand BMC at the baseline and its rate of loss over one year, analysis of covariance (ANCOVA) was used with presence of rheumatoid arthritis and menopause as two separate risk factors after correcting for confounders like age, height and weight. At baseline, both the presence of the disease ( $p < 0.0001$ ) and menopausal status ( $p = 0.007$ ) were important determinants of hand BMC however, for the rate of loss of hand BMC, menopausal status ( $p = 0.01$ ) and not presence of the disease ( $p = 0.8$ ) was the main determinant. In summary therefore, the changes in hand BMC in postmenopausal female patients are not solely disease dependent.

### **CONCLUSIONS AND COMMENTS**

1. Patients with rheumatoid arthritis had reduced hand bone mass than sex matched volunteers after correcting for age and body size.
2. Female patients had reduced hand bone mass than male patients after correcting for body size, age and disease duration.
3. There was no significant difference between the bone mass in the non-dominant and dominant hands in male or female patients.
4. Hand BMC inversely correlated with age in female patients only.
5. Hand BMC correlated inversely with disease duration, Larsen's and modified Sharp's scores in both, male and female patients.

6. The hand BMC in female patients with established RA is dependent on both, the menopausal state as well as the presence of the disease.
7. Over one year study period, there was no significant change in most of the parameters of disease activity except HAQ score and Larsen's score which worsened significantly.
8. Over one year period there was a wide variation in individual rate of change in hand BMC in patients although as a group, in patients with disease duration of more than two years, there was no change in hand BMC.

## **List of Figures**

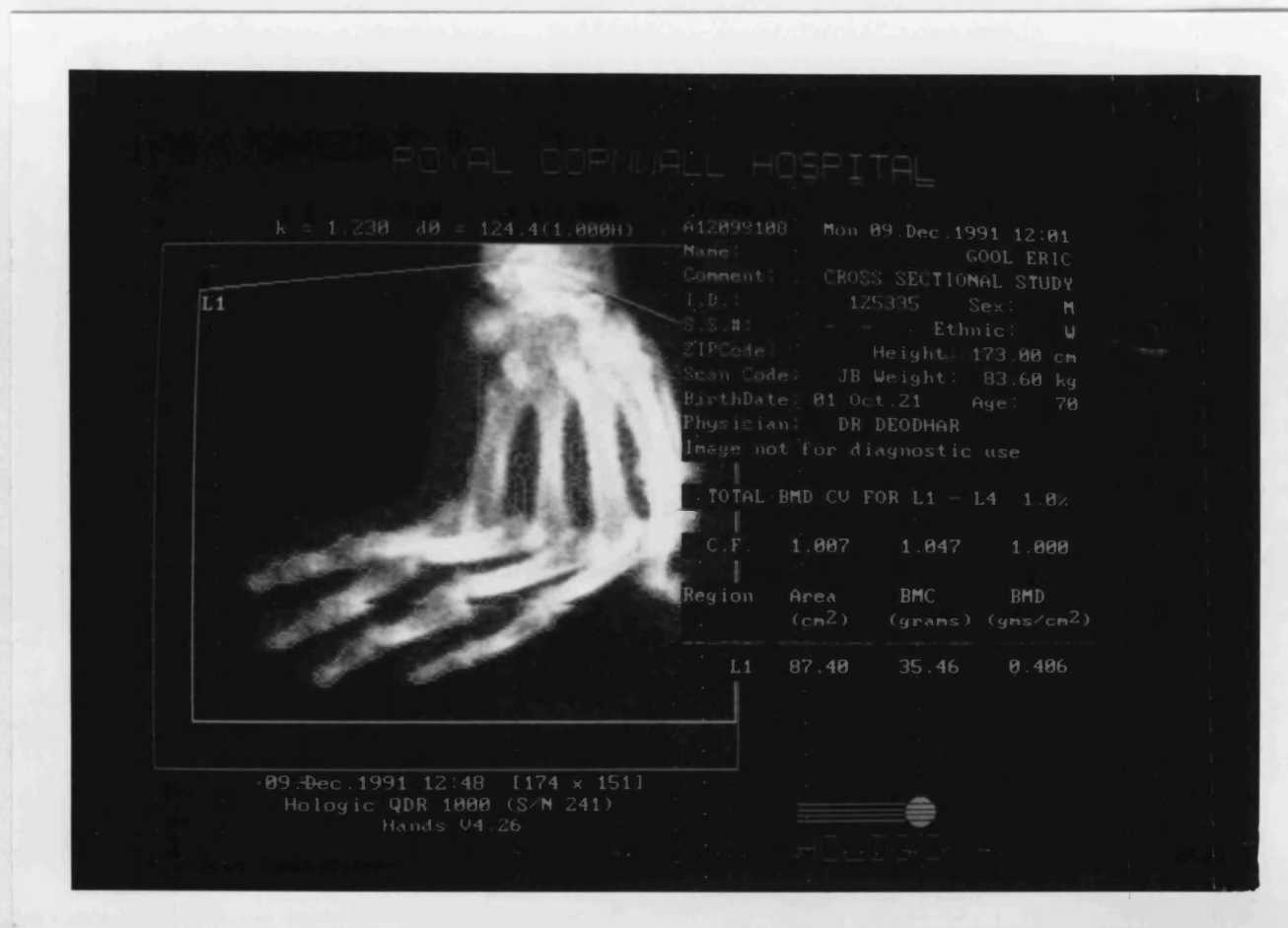
**Figure 1: Hand BMC in a patient with rheumatoid arthritis.**

**Figure 2: Hand BMC correlations with different disease parameters.**

**Figure 3: Comparison of hand BMC between normal volunteers and patients with RA.**

**Figure 4: Individual rate of change in hand BMC in patients with RA.**

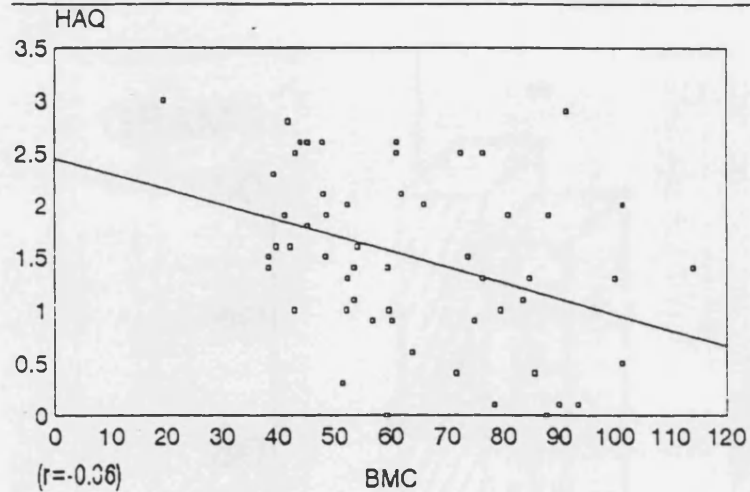
Figure 1: Hand BMC in a patient with rheumatoid arthritis.



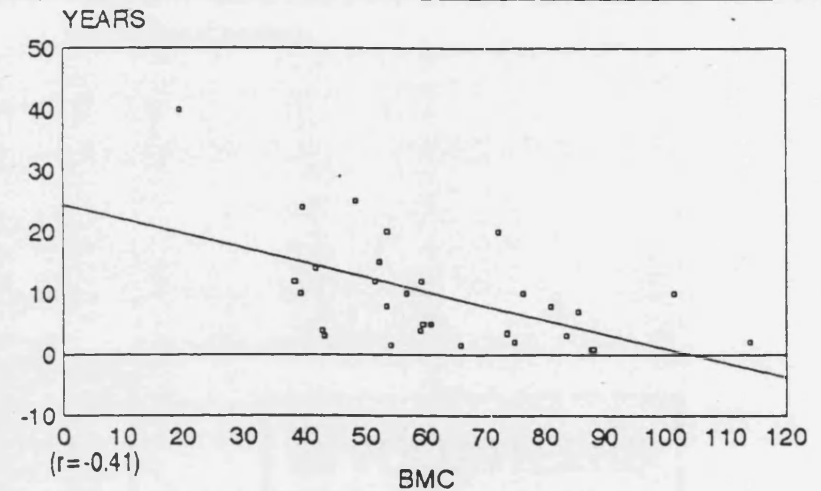


# HAND BONE MINERAL CONTENT (BMC)

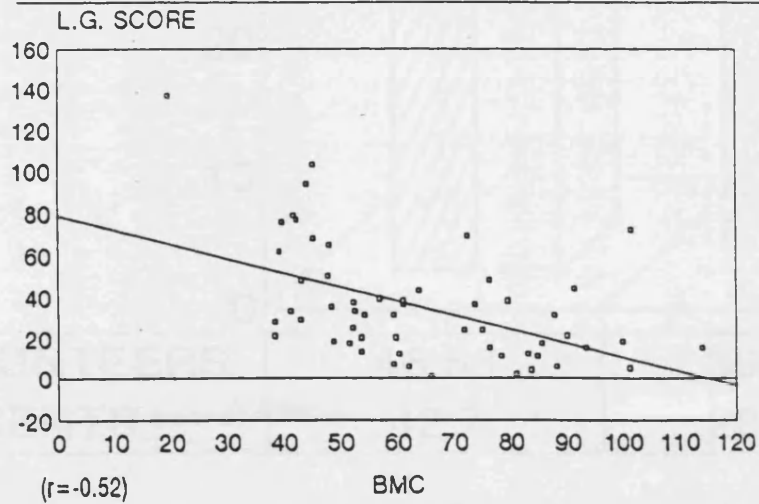
HAQ SCORE



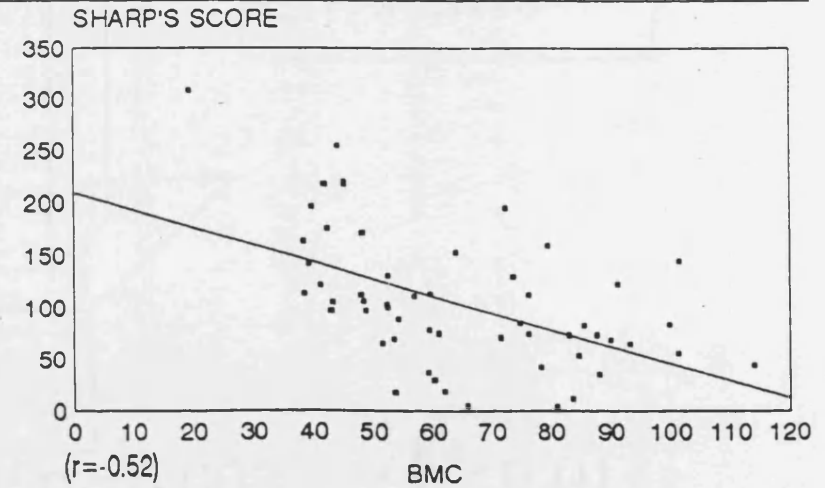
DISEASE DURATION\*



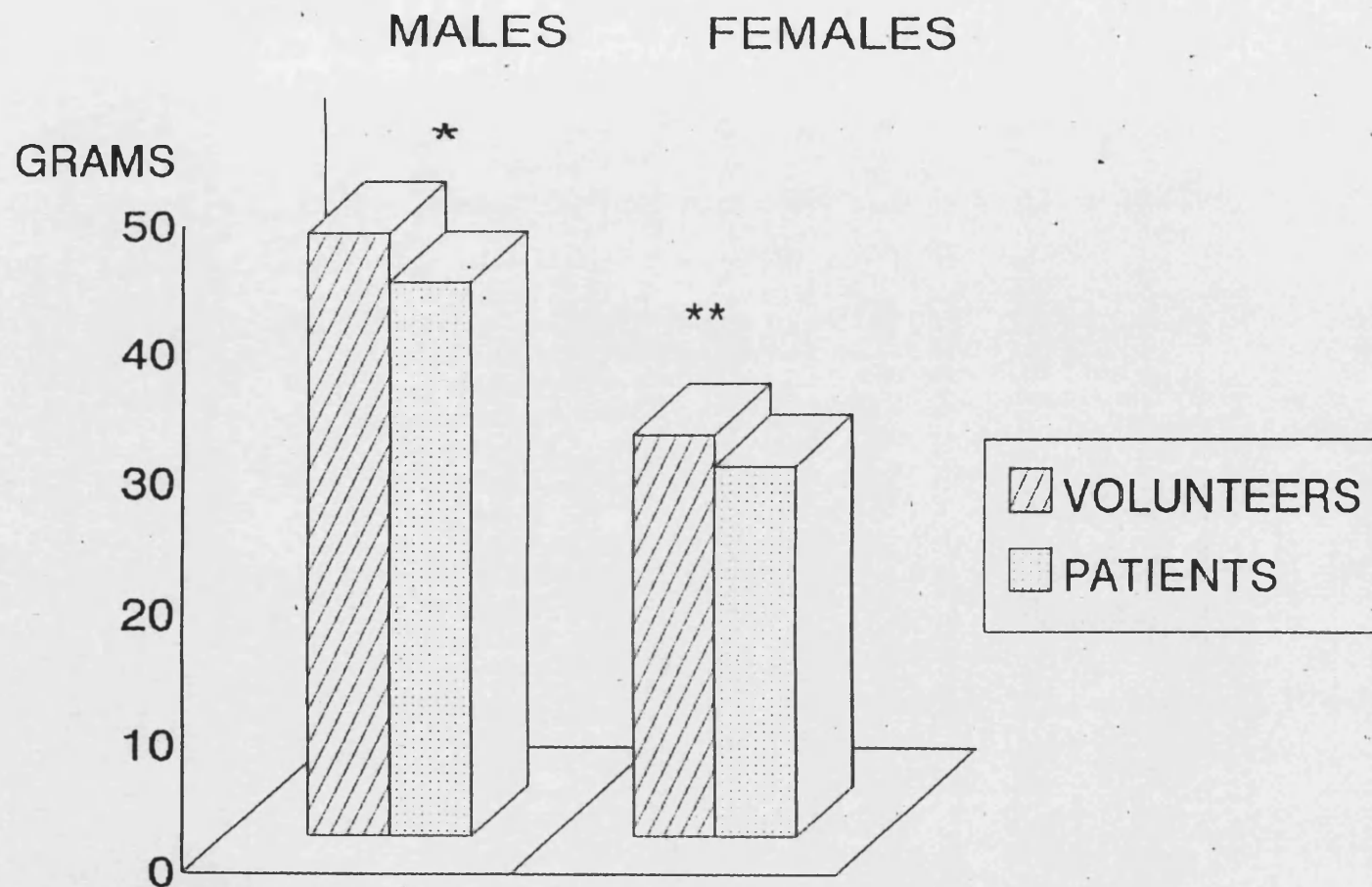
LARSEN'S GRADES



MODIFIED SHARP'S SCORE



# HAND BONE MINERAL CONTENT: DOMINANT HAND NORMAL VOLUNTEERS V PATIENTS WITH RA

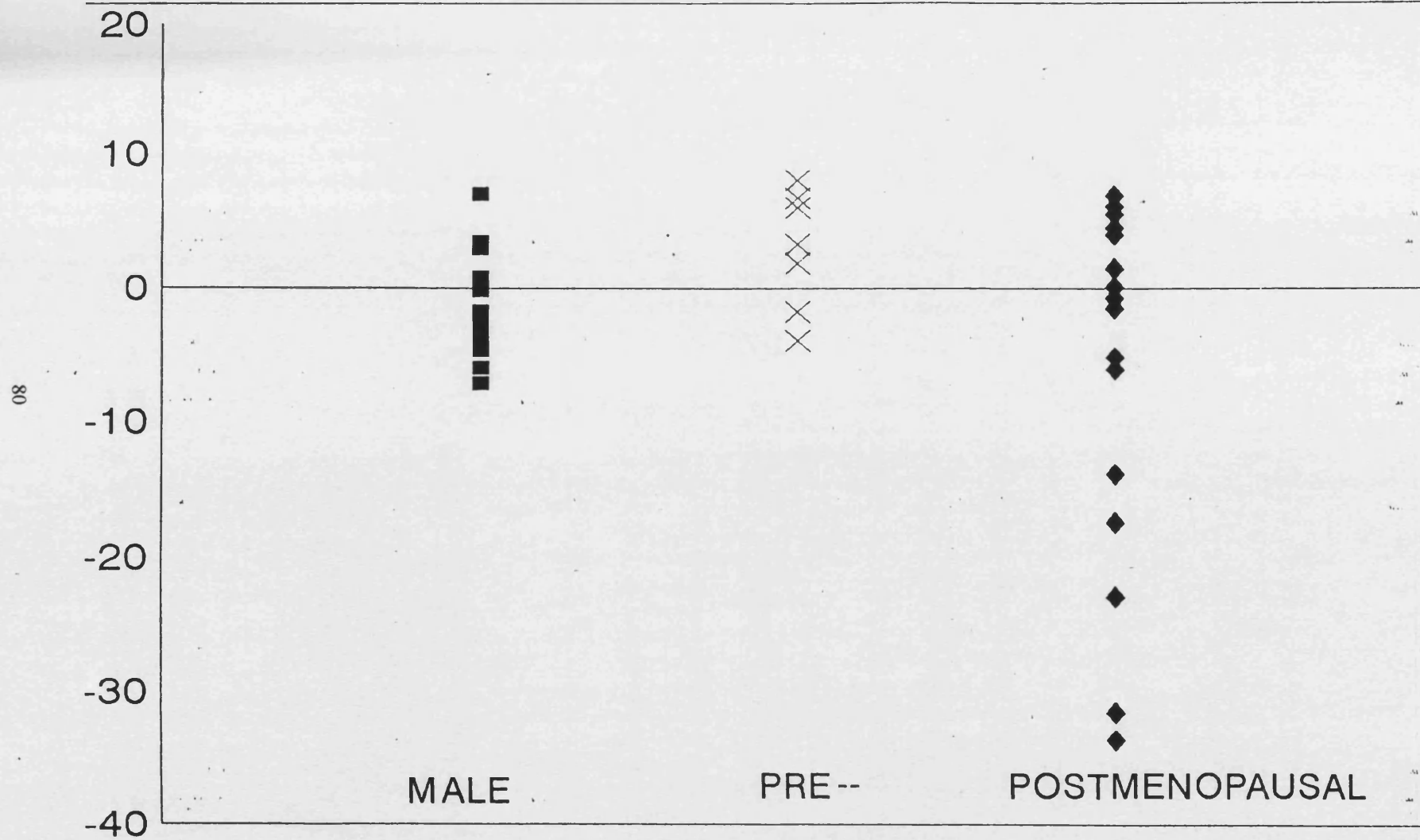


VOLUNTEERS	46.53	30.82
PATIENTS	42.7	28.41

\*  $p=0.003$     \*\*  $p<0.01$

# PERCENT CHANGES IN HAND BMC OVER ONE YEAR

PATIENTS WITH RA, DISEASE DURATION > 2 YEARS



**TWO YEAR FOLLOW-UP STUDY OF HAND BONE DENSITOMETRY AND BONE  
BIOCHEMISTRY IN EARLY RHEUMATOID ARTHRITIS**

## **INTRODUCTION**

In the last chapter, we have shown that patients with rheumatoid arthritis had reduced hand bone mass as compared to normal volunteers. However over one year period, we could not find any significant change in the hand BMC in this group of patients with established RA. One of the reasons for this lack of change in hand BMC could be the fact that most of these patients had a relatively long disease duration. Disease duration is an important factor to be considered when studying the effect of RA on bone mass. We therefore at the same time as performing the cross-sectional and longitudinal studies on patients with established RA, initiated an "Early RA Clinic" to study changes in hand BMC in patients with recent onset rheumatoid arthritis. Subsequently two other studies on axial skeleton in patients with RA (Gough et al 1994b, Shenstone et al 1994) have shown that the deleterious effect of RA occurs mainly in the first year of the disease and there were significant differences in the rate of loss of bone between patients with diseases duration of less than 1 year and those with disease duration of more than 1 year.

In this chapter, we describe a two year prospective study of changes in hand BMC in patients with early rheumatoid arthritis (disease duration less than two years). We have also studied the biochemical markers of bone metabolism over this period to assess mechanisms of and response to bone loss in rheumatoid arthritis.

## **SUBJECTS AND METHODS**

Forty three patient (17 males aged 35-77, median 53 years, and 26 females aged 29-71, median 55 years, 8 pre-menopausal) with early RA (disease duration median 9 months, IQR 10, range 2 to 22 months, diagnosed by ARA Criteria 1987), were selected consecutively from the out-patients and in-patients of the Duke of Cornwall Rheumatology Unit, Truro. None of these patients were taking any disease modifying anti-rheumatic drugs (DMARDs) at entry to the study, but during the study period, all except one were started on DMARDs (20 sulfasalazine, 8 penicillamine, 5 hydroxychloroquine, 3 sodium aurothiomalate intra-muscular, 3 pyritinol, 1 methotrexate and 1 patient received sodium aurothiomalate injection and sulfasalazine together). Patients with any other diseases likely to affect bone metabolism and female patients likely to be pregnant during the study period were excluded from the study. During the study period none of the patients received oral corticosteroids though three patients had

used oral corticosteroids in the past (at least 12 months before entry into the study) for other reasons. Informed written consent was obtained at the baseline visit.

The hand BMC data on the normal volunteers (control population) described in Chapter Four and in patients with longer duration of RA ("Late RA Group") described in Chapter five was used for comparison with these patients with shorter duration of RA (the "Early RA Group").

#### **Clinical Assessment and Bone Density Measurement:**

All patients were assessed at baseline and then at six monthly interval over the next two years. All 43 patients had at least three assessments (baseline, six months and one year). 39 patients had four assessments (up to month eighteen) and 29 patients completed five assessments (two years). At each visit a physical examination was performed. The clinical activity was determined by early morning stiffness (EMS), Ritchie articular index (Ritchie et al 1968) and swollen joint count. The general functional status was estimated by Health Assessment Questionnaire (HAQ) (Fries et al 1980) score, hand function was examined by grip strength and radiological severity was assessed on hand x-rays by Larsen score (Larsen et al 1977) by one observer. C reactive protein (CRP) and plasma viscosity was measured at each visit. BMC of the dominant hand was measured at each visit by DXA (Hologic QDR 1000) using a custom-built perspex-aluminium plate and locally modified spine analysis programme as described in the third chapter

#### **Biochemical Markers of Bone Metabolism:**

At each visit bone metabolism was assessed by urinary pyridinoline and deoxy-pyridinoline (reflecting osteoclastic activity (Eastell 1994)), serum osteocalcin and bone specific alkaline phosphatase (both reflecting osteoblastic activity (Eastell 1994)).

#### **Urinary Collagen Cross-links Excretion**

A two hour fasting urine collection was made by all patients at each assessment visit. The urinary excretion of pyridinoline and deoxy-pyridinoline is not affected by diet, though the same specimen of urine was also used for measurement of urinary calcium which can be diet dependent. The urine was collected in hospital in plain bottles, the volume was recorded and two 2 ml aliquots were stored at -20°C for further analysis.

The remaining urine was acidified with 10 ml of 10% HCl and was used for measurement of calcium creatinine ratio. The collagen crosslinks were measured using the method of Black et al (1988). One ml of urine was hydrolysed with one ml of concentrated HCl (with final concentration of 6 M) for 16 hours at 116°C. The resulting hydrolysates were fractionated by cellulose CFI column chromatography. The crosslinks were separated and quantified by reverse phase HPLC with fluorescent spectroscopy using heptafluorobutyric acid as an ion pairing agent. For each specimen duplicate samples were analyzed and from these the urinary excretion rates of the pyridinium cross-links were expressed as nmol/mmol of urinary creatinine. To determine the normal ranges for our laboratory, early morning urine samples were also collected from 57 normal volunteers (13 males, 36 pre and 8 post menopausal females, age range 20 to 75) who were different from the volunteers who took part in the hand scan experiment. None of these volunteers suffered from any conditions that could affect the bone metabolism and none were taking corticosteroids. For our laboratory, the intra-assay variation was 10% for pyridinoline and 15% for deoxy-pyridinoline. A control sample with known quantities of pyridinoline and deoxy-pyridinoline was included in each run for quality control.

### **Serum Osteocalcin Measurement**

Serum osteocalcin concentrations were determined using a commercially available radioimmunoassay (RIA) kit (CIS (UK) Ltd., High Wycombe, England). The test sensitivity has been determined to be 0.5 ngm/ml. The precision was assessed by using the control serum provided at regular intervals. For our laboratory the coefficient of variation (CV) of the method was found to be around 5%. Each determination was carried out in duplicate and samples giving higher CV were repeated for confirmation. The normal values (mean (SD)) for men were 6.5 (2.3), all women 4.8 (2.1) and for post-menopausal women 7 (3.7).

### **Estimation Of Bone Isoenzyme Of Alkaline Phosphatase**

The bone alkaline phosphatase activity was determined using the wheat germ lectin precipitation method described by Rosalki et al (1984) using a commercially available kit (Isoenzymes of alkaline phosphatase, Boehringer Mannheim UK). After the total activity of serum alkaline phosphatase was determined (using PNP AMP buffer, kit from Randox laboratories), bone alkaline phosphatase was precipitated using the lectin from wheat germ and the residual alkaline phosphatase activity was measured in the supernatant (Olympus AU 5000 machine). Taking the dilution factor into account, bone alkaline phosphatase activity was calculated as:

Bone Alk Phos =  $1.118 * \text{Total Alk Phos} - 2.35 * \text{activity in supernatant}$ . The normal values for our laboratory were total alkaline phosphatase upto 115 u/l and bone specific alkaline phosphatase upto 65 u/l.

## **ANALYSIS**

Number Cruncher Statistical System (NCSS) statistical programme was used for statistical analysis. Hand BMC of controls and patients was compared by Mann-Whitney test. Comparisons between controls and patients were made by carrying out an analysis of covariance with age, weight and height as covariates. Only those covariates which indicated a significant association with BMC were included in the final analysis. Baseline results in patients were compared with their results at month 6, 12, 18 and 24 by Wilcoxon matched pairs test. The percent change in hand BMC was calculated by comparing the results at every assessment visit with the baseline results. The annual percent loss was correlated with the mean values of variables by Spearman's correlation.

## **RESULTS AT BASELINE**

The demographic characteristics of the patients are shown in Table 1.

Table 1: Demographic Characteristics Of Patients (n=43) With Early RA

	<b>Male</b>	<b>Female</b>
<b>n=</b>	17	25
<b>Median Age</b>	53	55
<b>Age Range</b>	35-77	29-71
<b>Premenopausal</b>	--	8
<b>No. on DMARDS</b>	16	25
<b>Past steroid use</b>	0	3



## Disease Activity At Baseline

Table 2 shows the various parameters of disease activity at baseline.

Table 2: Baseline Disease Characteristics In 43 Patients With Early RA: Median (IQR)

Early Morning Stiffness	45 (40)
Swollen Joints (Hands)	4 (7)
Tender Joints (Hands)	14 (23)
Ritchie Score	14 (16)
Grip Strength Dominant Hand	27.3 (19)
Larsen Score	4 (8)
No. Of Erosions On Hand X-Rays	1 (3)
C Reactive Protein	17 (58)
Plasma Viscosity	1.84 (0.22)

## Hand Bone Mineral Content At Baseline

At the initial assessment, male and post-menopausal female patients had significantly lower hand BMC than controls after correcting for age, height and weight (Table 3, male patients 7% reduction,  $p=0.04$ , postmenopausal female patients 8.5% reduction,  $p=0.04$ ). The premenopausal female patients had higher hand BMC than controls, but it was not statistically significant.

Table 3: Hand bone mineral content in patients and controls (in grams) at baseline

	Control		Patients	
	Median	95% CI	Median	95% CI
Male	46.53	44.7-47.9	43.49	42.6-44.3
Pre-menopausal	31.57	30.2-35.1	34.0	28.9-40.5
Post-menopausal	30.12	29.3-30.9	27.76	26.9-28.5

### **Biochemical Markers of Bone Metabolism At Baseline:**

At baseline, serum osteocalcin was significantly higher in female ( $p=0.0004$ ) but not in male patients as compared to normal controls. Urinary excretion of pyridinoline/creatinine (Pyd/Cr) and deoxy-pyridinoline/creatinine (Dpd/Cr) were significantly elevated in male and female patients as compared to controls at baseline (for males: Pyd/Cr  $p=0.005$ , Dpd/Cr  $p=0.01$ ; for females: Pyd/Cr  $p=0.001$ , Dpd/Cr  $p=0.003$ ). The serum levels of bone isoenzyme of alkaline phosphatase was in the normal limits, both in male and female patients.

### **RESULTS: TWO YEAR FOLLOW-UP**

#### **Disease Activity On Follow Up**

Over the two year study duration, patients showed overall improvement in disease activity with fall in EMS, Ritchie articular index, swollen joint count, tender joint count, plasma viscosity, CRP and improvement in grip strength. The Larsen score and the number of erosions on hand x-rays progressively worsened over two year period. There was no significant change in the HAQ score (for detailed results see Table 4, Figure 2).

**Table 4: Percent Changes (From Baseline) In Disease Characteristics In Patients Over Two Years: Non-parametric Wilcoxon matched pairs test, median (IQR).**

<b>Paired Differences In Variables</b>	<b>Baseline Value</b>	<b>Percent Change At One Year</b>	<b>P* Value</b>	<b>Percent Change At Two Years</b>	<b>P* Value</b>	<b>P** Value</b>
Early Morning Stiffness	45 (40)	-33.3 (140)	0.04	-77 (95)	0.01	0.18
Swollen Joints (Hands) <sup>⊗</sup>	4 (7)	-66.6 (100)	0.001	-50 (117)	0.003	0.5
Tender Joints (Hands) <sup>⊗</sup>	14 (23)	-38 (103)	0.007	-46 (78)	0.0003	0.2
Ritchie Score	14 (16)	-28 (75)	0.01	-39 (60)	0.0002	0.16
Grip Strength Dominant Hand	27.3 (19)	39 (104)	0.001	48 (88)	0.004	0.4
Larsen Score	4 (8)	64 (300)	0.0000	100 (285)	0.0001	0.04
No. Of Erosions (X-Ray: Both Hands)	1 (3)	66.6 (213)	0.0003	230 (358)	0.0002	0.003
C Reactive Protein	17 (58)	-35 (91)	0.01	-41 (189)	0.5	0.4
Plasma Viscosity	1.84 (0.22)	-5 (7.6)	0.0001	-4 (12.8)	0.009	0.3
Osteocalcin <sup>⊖</sup>	7.1 (3.8)	15 (53)	0.02	18 (77)	0.02	0.8
Bone Alk. Phos <sup>⊗</sup>	15 (17.4)	32 (116.6)	0.006	12 (144)	0.3	0.19
% Bone Alk Phos <sup>†</sup>	16.2 (16.9)	46 (139)	0.001	18.6 (174)	0.4	0.38
Urinary Pyridinol/creatinine		-16.6 (112)	0.66	-25.6(148)	0.79	0.32

Note: \*Comparison with baseline value, \*\*Comparison of one year value with two year value, <sup>⊗</sup> Total number of swollen joints in hands, <sup>⊖</sup> Total number of tender joints in hands, <sup>⊖</sup> nanograms per ml, <sup>⊗</sup> units per litre. <sup>†</sup>Percentage of bone isoenzyme in total alkaline phosphatase.

#### **Rate Of Change In Hand BMC Over Two Years In Patients**

Both male and female patients lost significant amount of hand BMC (Table 5, Figure 3) over two year period despite improvement in the parameters of disease activity. For males, the percent change in hand BMC (median (IQR)) compared to baseline result was: at six months -6 (7.3), at 12 months -4.8 (7.5), at 18 months -9 (9.1) and at 24 months -4.8 (8.2) and for females it was: at six months -2.6 (5.7), at 12 months -3.5 (10.5), at 18 months -7.1 (8.2) and at 24 months -6.5 (13.7). The comparison of results at 18 months with those at 24 months showed that female patients had regained 0.7 (7.6) percent but male patients had lost 0.1 (7.3) percent hand BMC over the last six months, however these changes did not reach statistical significance.

**Table 5: Percent Changes (From Baseline) In Hand BMC In Patients With Early Rheumatoid Arthritis Over Two Years. Non-parametric Wilcoxon matched pairs test, median (IQR).**

<b>Paired Differences In Variables</b>	<b>Baseline Value In Grams</b>	<b>Percent Change At One Year</b>	<b>P* Value</b>	<b>Percent Change At Two Years</b>	<b>P* Value</b>	<b>P** Value</b>
Total Group	34.3 (15.1)	-4.65 (8.5)	0.0001	-6.4 (11.2)	0.0000	0.02
Males	43.5 (7.27)	-4.8 (7.5)	0.005	-4.8 (8.2)	0.001	0.08
All Females	28.7 (5.7)	-3.5 (10.5)	0.009	-6.5 (13.7)	0.002	0.1
Pre-Menopausal Females	34.0 (6.6)	-4.6 (8.2)	0.1	-6.3 (6.9)	0.03	0.7
Post-Menopausal Females	27.7 (4.5)	-3.2 (11.3)	0.04	-8.9 (14.2)	0.02	0.2

Note: \*Comparison with baseline value, \*\*Comparison of one year and two year values.

### **Changes In The Biochemical Parameters Of Bone Metabolism**

There were significant increases in the serum levels of osteocalcin ( $p=0.02$ ) and bone specific alkaline phosphatase ( $p=0.006$ , Table 3, Figure 4) in the first year but no significant changes over the second year. There was a significant increase in the percentage of bone isoenzyme in total alkaline phosphatase over the first year ( $p=0.001$ ) and a similar trend over the second year ( $p=0.05$ ). Throughout the two year period, urinary excretion of pyridinoline/creatinine (Pyd/Cr) and deoxy-pyridinoline/creatinine (Dpd/Cr) remained significantly elevated compared to normal ranges in females (for Pyd/Cr at 12 months  $p=0.006$ , at 24 months  $p=0.008$ ; for Dpd/Cr at 12 months  $p=0.02$ , at 24 months  $p=0.0009$ ) but only for the first year in males (at 12 months, Pyd/Cr  $p=0.003$  and Dpd/Cr  $p=0.02$ ). Compared to baseline results, however there was no significant change in either Pyd/Cr or Dpd/Cr excretion in male or female patients.

These biochemical parameters of bone metabolism however did not correlate with loss of hand BMC.

### **Hand BMC Correlations With Other Parameters Of Disease Activity And Severity**

Over the two year study period, the percent annual loss of hand BMC correlated with mean number of swollen joints in the whole body ( $r=0.35$ ,  $p=0.02$ ), mean plasma viscosity ( $r=0.33$ ,  $p=0.02$ ), mean CRP ( $r=0.31$ ,  $p=0.04$ ) and mean number of swollen

joints in both hands ( $r=0.3$ ,  $p=0.04$ ). The percent annual loss of hand BMC also correlated with percent increase in Larsen score ( $r=0.36$ ,  $p=0.03$ ).

### **Effect Of Menopause On Hand BMC**

To investigate whether menopausal status affected the hand BMC at the baseline and its rate of loss over one year, analysis of covariance (ANCOVA) was used with presence of rheumatoid arthritis and menopause as two separate risk factors after correcting for confounders like age, height and weight.

In female patients with early RA, menopausal status ( $p=0.0001$ ), but not the presence of disease ( $p=0.1$ ), was the important determinant of hand BMC at baseline but, presence of rheumatoid arthritis ( $p=0.03$ ) and not the menopausal status ( $p=0.2$ ) was the sole determinant for the rate of loss of hand BMC.

In summary therefore, presence of rheumatoid arthritis is the sole determinant of the rapid loss of hand BMC in females with early RA.

### **COMPARISON BETWEEN CONTROLS, EARLY AND LATE RA GROUPS: CHANGES IN DOMINANT HAND BMC OVER ONE YEAR**

We compared the changes in the dominant hand BMC in the normal volunteer control group (Chapter 4) and in the established RA group ("Late RA", Chapter 5) over one year period with the similar changes in the "Early RA" group. At baseline, there was no significant difference in the hand BMC in males in early and late RA groups after correcting for age ( $p=0.1$ ). The rate of loss of hand BMC in males in the early RA group however was significantly greater than in the males from late RA group ( $p=0.01$ , Table 6, Figure 5) after correcting for age.

**Table 6: Percent Change In Dominant Hand BMC Over One Year In Controls And In Patients With RA**

<b>GROUPS:</b>	<b>n=</b>	<b>MEDIAN</b>	<b>IQR</b>	<b>*p=</b>
<b>CONTROLS</b>				
Males	9	0.9	1.73	0.2
Females	28	-0.9	1.92	0.08
<b>LATE RA</b>				
Males	16	-2.24	4.33	0.1
Females	23	0.11	11.56	0.2
<b>EARLY RA</b>				
Males	17	-5.26	11.49	0.007
Females	25	-2.14	10.1	0.03

Note: \*p value refers to the paired differences between hand BMC at entry and at one year by Wilcoxon Matched Pairs Test.

Female patients from the late RA group had significantly lower hand BMC than female patients in the early RA group after correcting for height, weight and age (at entry  $p=0.0004$  and at one year  $p=0.01$ , Table 5, Figure 5). Females in the early RA group lost more bone than the females in the late RA group, but this difference in the rate of loss of hand BMC did not reach statistical significance.

## **CONCLUSIONS AND COMMENTS**

1. At initial assessment, male and post-menopausal female patients with early RA had significantly reduced hand bone mass as compared to normal volunteers.
2. Patients with early RA lost hand BMC progressively over eighteen months despite clinical improvement.
3. This bone loss correlated with mean values of number of swollen joints in body, number of swollen joints in hands, plasma viscosity and the CRP.
4. The biochemical markers of bone resorption (pyridinoline cross-links) were increased in patients at the start and remained increased throughout the study period.
5. The biochemical markers of bone formation were increased over the first year.
6. These findings suggest that the clinical improvement as shown by the reduction in the disease activity, combined with the increased osteoblastic activity led to stabilization of the hand bone mass at two years.
7. In female patients with early RA, the presence of the disease (but not the menopausal status) was the sole determinant of the loss of hand BMC.
8. The annual rate of loss of hand bone mass was greater in patients with early RA when compared to patients with late RA.

## **List of Figures**

**Figure 1A and 1B: Measurement of hand bone mineral content (BMC) in a patient with early RA at entry (1A) and at 12 months (1B). Note the loss of 4.6 grams (9.6%) in hand BMC over one year period.**

**Figure 2: Changes in disease parameters in patients with RA over two years.**

**Figure 3: Changes in hand BMC in patients with RA over two year period. Note: M = Males, Pre = Premenopausal Females, Post = Postmenopausal Females.**

**Figure 4: Changes in biochemical parameters of bone metabolism in patients with RA over two year period.**

**Figure 5: Changes in hand BMC over one year in controls and patients. Note: 1 = Male Volunteers, 2 = Male Patients: Early RA, 3 = Male Patients: Late RA, 4 = Female Volunteers, 5 = Female Patients: Early RA and 6 = Female Patients: Late RA.**



Figure 18

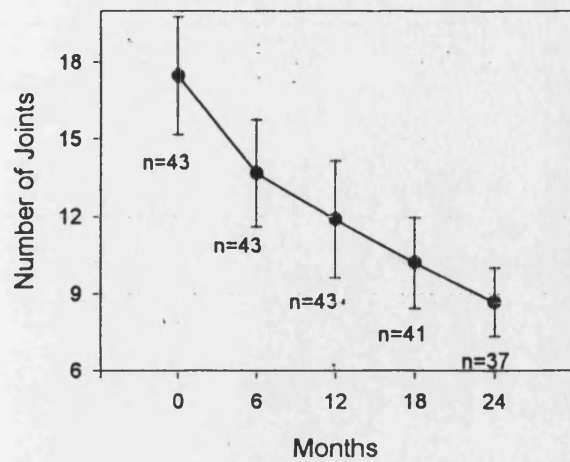
Figure 3: Hand position: flat.



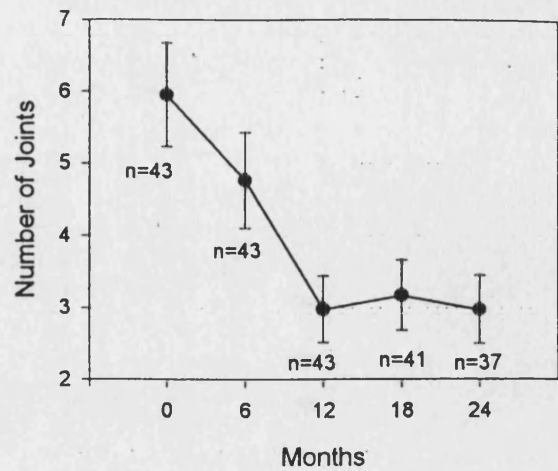
Figure 1B



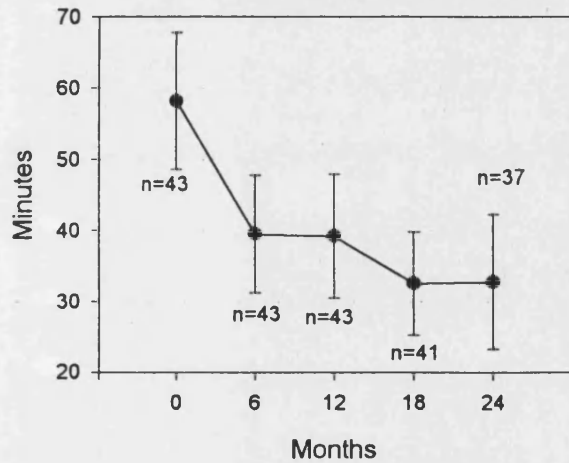
Number of Tender Joints in Both Hands



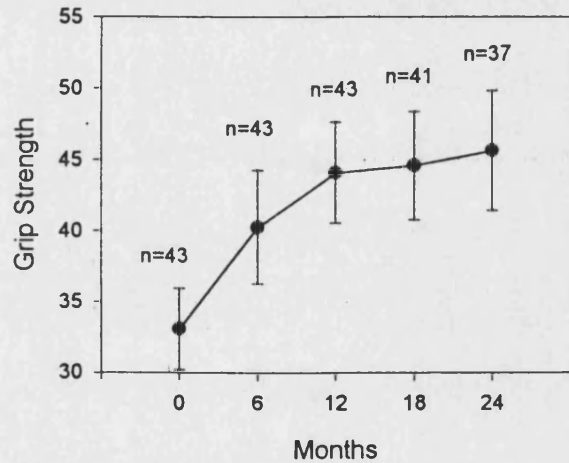
Number of Swollen Joints in Both Hands



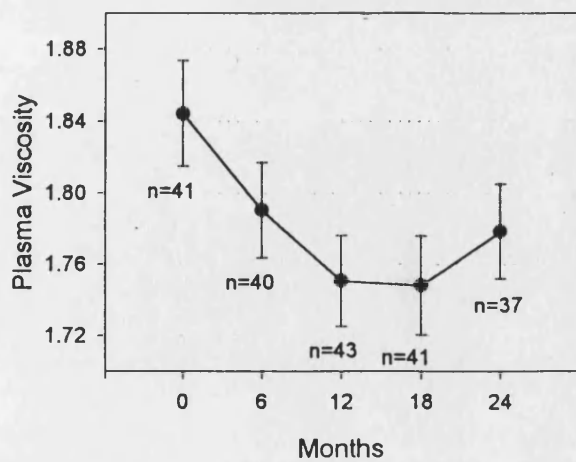
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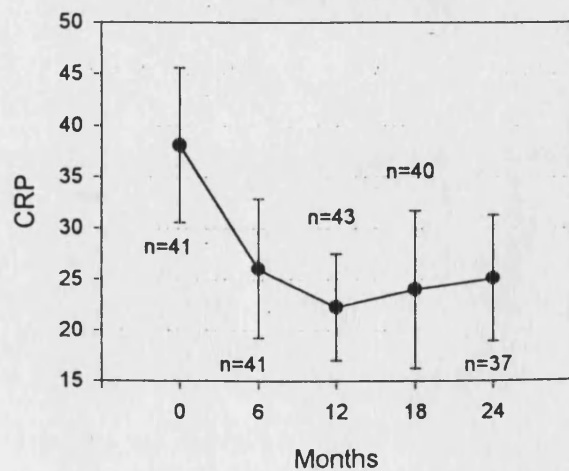
Dominant Hand Grip Strength



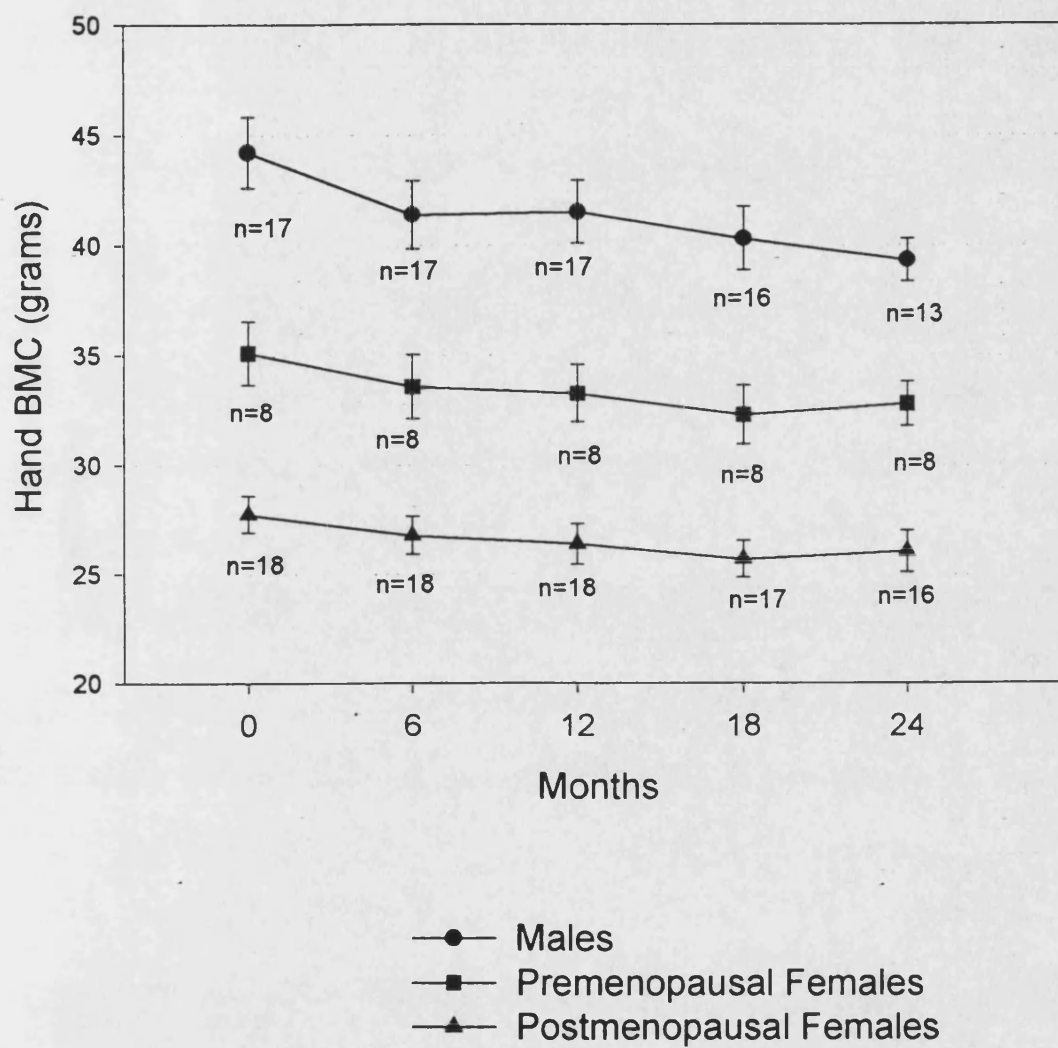
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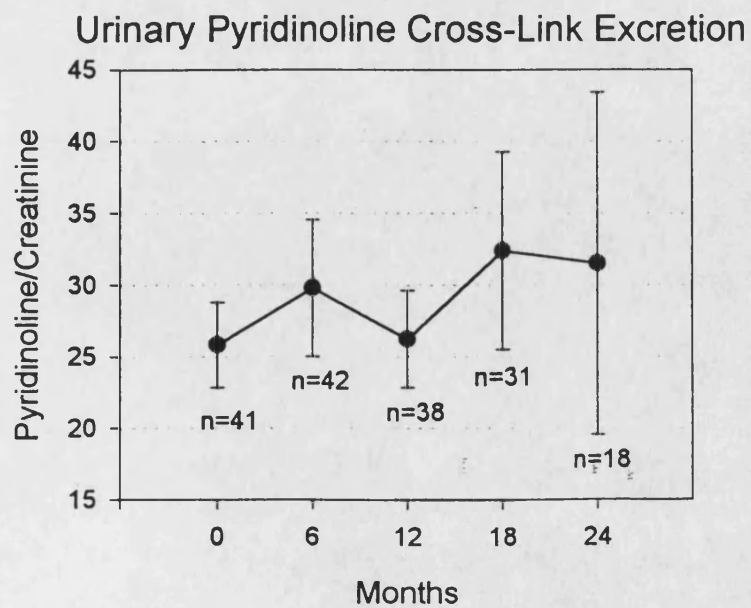
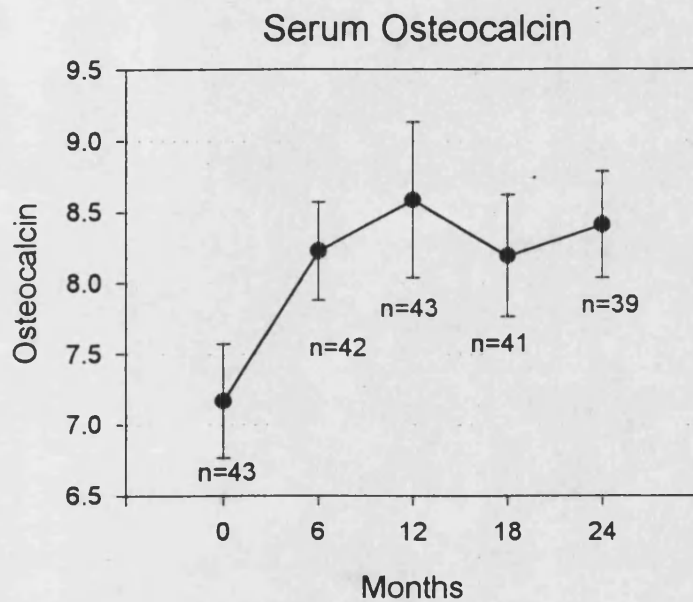
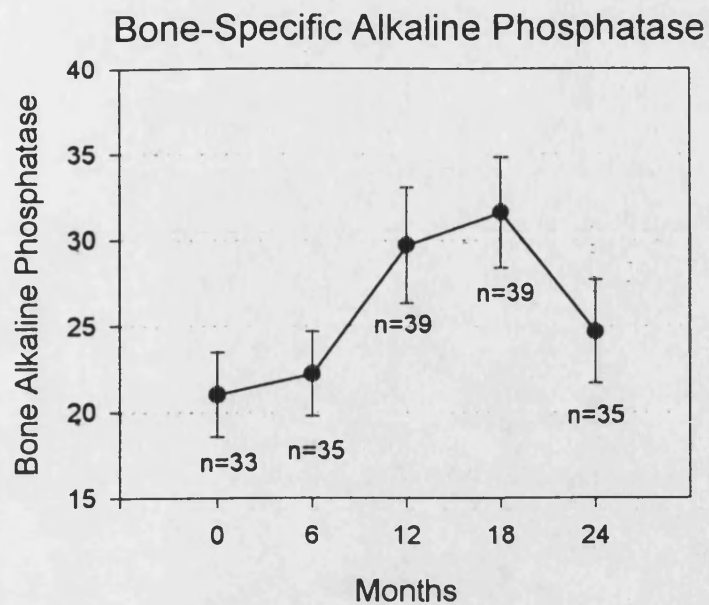


C Reactive Protein



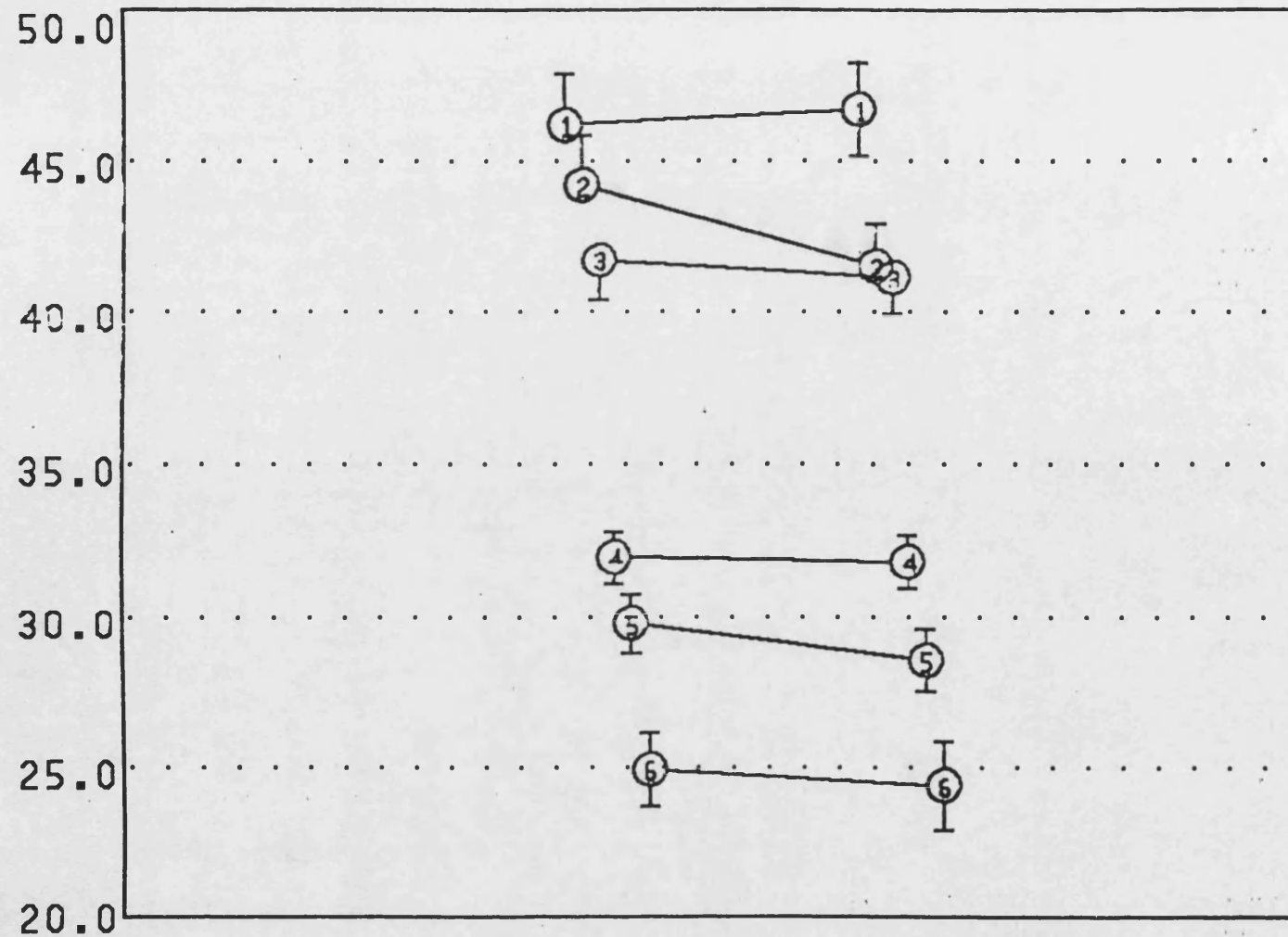
## Change in Hand BMC over 2 Years





# CHANGE IN HAND BMC OVER ONE YEAR

GRAMS



## **DISCUSSION AND CONCLUSIONS**

We have developed a reproducible method of measuring hand bone mineral content using dual energy x-ray absorptiometry, by modifying the existing protocol of measuring BMC in the spine.

When scanning the spine, the greater bulk of the soft tissue "hardens" the x-ray beam by removing the lower energy x-rays. The Hologic QDR 1000 dual energy x-ray absorptiometer is therefore dependent on the soft tissue thickness (STT) and is accurate and precise to measure the BMC in the spine at that level of x-ray beam hardness. The hand has a much smaller amount of soft tissue compared to the spine and hence to get accurate and precise results for measurement of hand BMC, the Hologic spine protocol had to be altered.

Pye et al (1990) described a method of scanning the hand to overcome the problem of the dependency of spine protocol on the soft tissue thickness. Their approach was to artificially move the hand bone mass and the soft tissue towards a region where the response would be less sensitive to the STT. They suggested to use a perspex aluminium plate for this purpose. This plate can compensate for the smaller tissue bulk found in the hand so as to increase the hardness of the x-ray beam to match that when scanning the spine. We assessed three protocols of hand bone densitometry, two of which did not use such a plate. However, these were found to be less precise when dealing with an osteoporotic hand. We therefore decided to use a plate of 29 mm thickness (7mm aluminium and 22 mm of perspex) which was found to be adequate for our machine (Protocol 1), though further experiments are required to design an optimum thickness plate which can be used on other machines.. Use of this plate reduced the accuracy as the results were consistently higher than the ashed weight. We however think that this is unlikely to cause a major problem in clinical practice, as change in hand BMC in longitudinal studies is going to be more important than the absolute value in an individual.

Calibration factors Q1, Q2 and Q3 are used to obtain a linear relationship between the actual amount of bone mineral and the result obtained by the DXA scan. These exist for the hip and spine but had to be calculated for the smaller bones of the hand. This was achieved by scanning an aluminium step wedge of known different thickness to mimic the different quantities of bone mineral within the small bones of the hand.



Our method gave a measurement of hand BMC with a coefficient of variation of 2.3% with no additional inter-observer variation. As RA progresses, the consequent deformity results in a change in hand position. However, our reproducibility data shows that the precision of measuring total hand BMC was unchanged by the hand position. In contrast, BMD measurement depends on the area of the hand, which is altered by changing the hand position. Bone mineral content rather than bone mineral density should therefore be used for further longitudinal studies of the hand. This method takes 10 minutes to scan one hand and a further 3 to 4 minutes to analyze it. All volunteers found the position of scanning quite comfortable. Patients with rheumatoid involvement of the shoulder with poor abduction found most difficulty positioning the hand on the machine but by adjusting the height of the chair they were able to find a satisfactory position for the duration of the scan.

Most of the analysis is automated with automatic delineation of the bones of the hand minimizing operator interaction and consequent variability. Change of hand position from flat to slightly flexed, simulating rheumatoid hand, did not alter the precision of the measured hand BMC whereas a slight change in hand position can alter the assessment of joint space and erosions by plain radiographs (Brower 1990) which are conventionally used to monitor progression of RA. This method is therefore more objective than plain radiographs and avoids the subjectivity of x-ray scoring methods.

Hand BMC correlated in both sexes with indices of body size like forearm span, height, weight and hand volume. In volunteers it did not correlate with age in contrast to the BMC in spine or hip, which is known to reduce after menopause in females. This apparent difference in age related changes to other parts of the skeleton could be explained by the "regenerative changes" of osteoarthritis. Some of the classical changes of osteoarthritis include sclerosis of bone margins and development of new bone such as the Heberden's and Bouchard's nodes in hands. In DEXA measurements of the spine, the osteophyte formations coupled with the sclerotic changes are known to give higher results of BMC (Masud et al 1993). None of our volunteers had clinical evidence of osteoarthritis but asymptomatic osteoarthritis in hands is quite common with advancing age. We did not however perform plain hand x-rays of our volunteers.

Exercise is known to stimulate bone growth (Bouxsein et al 1994) but our study found no difference between the BMC of the dominant and non-dominant hand in the volunteers. They were not however manual workers but principally hospital staff.

Radiological changes in the hands have been used for many years to assess the outcome of RA but significant problems exist in quantifying them (Brower 1990). The global scoring method of Larsen (Larsen et al 1977), where a single score is given for all the radiological abnormalities in the joint under consideration, and the detailed scoring method of Sharp (Sharp et al 1985), where a separate score is given to each radiological abnormality in the joint concerned, have the problem of poor sensitivity to monitor change. Change of hand position can alter the assessment of joint space and erosions by plain radiographs (Brower 1990). These methods can therefore be either imprecise and coarse or too cumbersome and time consuming. They require interested and trained observers. Microfocal radiographs and MRI scans can detect changes earlier than conventional radiography, though the changes in microfocal radiographs are not quantitative and in the case of MRI, the costs could be prohibitive. In contrast, measurement of hand BMC is quantitative, reproducible even on changing hand position and automated to avoid the observer error. It is much cheaper than an MRI scan and can be performed on machines easily available in district general hospitals.

The subject of generalized osteoporosis in inflammatory arthritis has been extensively studied though most of the studies have measured bone density at the lumbar spine and femoral neck to examine the effect of inflammation at these distant sites (Bhalla et al 1992, Laan et al 1992, Sambrook et al 1985, Gough et al 1991). As the hip is not always and lumbar vertebrae are very rarely involved directly by the disease process in RA, the changes in BMC at these sites largely indicate the generalized changes in bone metabolism caused by an inflammatory disorder. Our method however is not aimed at only assessing these more generalized changes but principally assessing what is happening at a site of widespread disease involvement and of functional impairment. The hand with its multiple joints and periarticular bone involvement in RA combined with the functional effects of disease on its usage may act as a site for composite assessment of overall disease progression by measurement of the bone mineral content.

After correcting for physical size, patients (male and female) with early RA had on average 7.6% lower hand bone mass even at entry as compared to controls and on average lost a further 2.35% hand BMC over one year. Rheumatoid arthritis is known to

cause a generalized skeletal effect with bone loss in femoral neck and lumbar spine (Shenstone et al 1994, Gough et al 1994) though a recent cross-sectional study has shown that changes in hand bone mass are more dramatic than changes at other sites (Peel 1994), with a reduction in hand bone mineral density of 23% as compared to 16% at the femoral neck and 11% at the lumbar spine (Peel et al 1994). The loss of hand bone mass in patients with RA therefore appear to be a combined result of the generalized plus local effects of the disease.

There are several possibilities why patients with RA had significantly lower hand BMC. Certain cytokines like Interleukin 1, Interleukin 6 and tumour necrosis factor  $\alpha$  which are potent stimulators of bone resorption, have been implicated in the juxta-articular as well as generalized bone loss in rheumatoid arthritis (Bhalla et al 1993). Treatment with corticosteroids and reduced mobility have also been suggested as possible important factors (Laan et al 1992, Riis et al 1985) but in this study we could not find any appreciable effect of steroid use although the numbers were small. Menopausal status appears to be a co-factor for loss of hand BMC in postmenopausal females with RA of longer duration but the rapid loss of hand BMC in early RA is independent of the menopausal status. Hormone replacement therapy (HRT) has recently been shown to increase spinal bone density and maintain femoral bone density (Hall et al 1994) in patients with RA. It would be interesting to see whether HRT has a similar protective role on hand bone loss.

In patients with RA, hand BMC correlated variably with parameters of disease severity but not with parameters of disease activity. Hand BMC did not show significant correlation with Ritchie Index, swollen joint count or duration of early morning stiffness, loose indicators of ongoing inflammatory disease process. Correlation was however better with disease duration and with the radiological scores. It is not surprising to find the poor correlation between the measures of disease activity with a single measurement of hand BMC as logically one would expect hand BMC to be an outcome measure and the better correlation with the disease duration and radiological measures of disease outcome might be expected.

Our longitudinal data demonstrates that the most rapid rate of loss of hand bone density occurs very early in the disease (over one year 4.88% in early RA males and 2.14% in early RA females). Studies looking at the bone density in the lumbar spine and femoral neck of patients with RA have also shown significant bone loss very early in the disease

(Shenstone et al 1994, Gough et al 1994). Long term follow-up in these patients is needed to see whether the loss of hand BMC correlates with future loss of function and poor outcome. The annual rate of change of hand BMC in patients with early RA varied from +11% to -22% and this correlated with baseline CRP but did not correlate with indicators of hand function such as grip strength. This loss occurred despite improvement in other validated measures of disease activity indicating that even though the current treatment of RA is successful in improving symptoms and markers of inflammation, it may not be very effective in controlling the early loss of bone. However, there appears to be a lag period of about 18 months before these benefits are reflected in hand BMC measurement. The data from the second year of our prospective longitudinal bone densitometry study showed that by suppressing the disease activity effectively the process of bone loss can be halted. We therefore think that our study provides yet another strong reason to intervene as early as possible to prevent the early rapid bone loss in hands in patients with RA.

Urinary collagen cross-link excretion remained high over most of the time in our patients indicating continued degradation of collagen from bone and cartilage. However, the biochemical markers of osteoblastic activity (osteocalcin and bone isoenzyme of alkaline phosphatase) showed significant increases in the first year of the study and in case of bone alkaline phosphatase, remained elevated even in the second year (although did not reach statistical significance due to small numbers at the end of two years). It therefore appears that the compensatory osteoblastic reaction could be responsible for stabilisation of the hand BMC later in the disease course. Previous studies on serum osteocalcin (OC) in patients with RA have demonstrated variable results (Sambrook et al 1985, Weisman et al 1986, Ekenstam et al 1986, Magaro et al 1989) but two other studies have shown raised OC levels in patients with RA (Magaro et al 1989, Marhoffer et al 1991) which were found to correlate with the disease activity.

Larsen score and number of erosions also worsened significantly in our study and the increase in Larsen score correlated with loss in hand BMC. However, as explained above, the Larsen's method of scoring radiographs is cumbersome, time consuming and needs interested and trained observers. In inexperienced hands, early radiographic changes can be subjective, resulting in poor reproducibility. Nearly two decades after it was first described, it has therefore remained a research tool. Hand BMC measurement has the advantage of being quick, precise and has no inter-observer variability. It also has the potential to be performed routinely on standard equipment available in many hospitals. Our experience with hand BMC measurement in patients with RA shows that

this technique could bridge the gap between a 'process' and an 'outcome' variable as it shares properties of both.

Hand bone densitometry is an accurate, reproducible and sensitive quantitative measure of bony changes in the hand that has the potential to be used as a gold standard outcome measure in early stages of rheumatoid arthritis, the very crucial time when maximum damage to the bones is done. It also opens up new possibilities of evaluating newer therapies aimed to prevent these early bone changes and hand bone densitometry will be the investigation of choice of monitoring them. The future of this method lies in longitudinal studies to see whether the early loss of hand bone mass in patients with RA can predict erosions and subsequent disability and whether early pharmacological interventions can prevent this bone loss. Further long-term studies are required to clarify whether this rapid and early loss is clinically relevant to functional outcome.

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